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Biological Sex on the Therapeutic Effectiveness of Various Treatments of Major Depressive Disorder

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ABSTRACT

Major Depressive Disorder (MDD) is one of the most prevalent mood disorders in the world. Anatomical differences between male, female, heterosexual, and homosexual individuals such as the hypothalamus and hippocampus cause for larger prevalence of the disorder in females and homosexuals. The purpose of this study was to elucidate the effect of biological sex and sexual orientation on different treatments of MDD. The model organism, *Drosophila melanogaster*, was used due to its human gene homologs of the *5HT1A, fru,* and *dsx* genes. The *5HT1A* gene mutation decreases serotonin which is linked to MDD. The *fru* gene mutation was used to model homosexuality and the *dsx* gene mutation was used to model transgender identities. Double and triple cross mutants were made through careful genetic crosses to create a *fru*, *dsx*, and 5-HT1A mutant. The hypothesis was, if males, females, *fru* mutants, *dsx* mutants, 5HT1A mutants, and combinations of those received different treatments for MDD, then their effectiveness will vary based on the genetic profile of the mutants. The popular antidepressants fluoxetine, sertraline, and lithium chloride were tested as treatments. Behavioral and biochemical tests tested symptoms of MDD such as decreased appetite, decreased activity, decreased serotonin levels. The hypothesis was supported, results showing that variable groups experienced different responses to different treatments. Results demonstrated that lithium chloride was most effective in males, fluoxetine most effective in females, and sertraline most effective in homosexuals. Therefore, drug treatments differ amongst individuals of differing sex and sexual orientation and should be explored in future experiments.

Introduction

Major Depressive Disorder (MDD) is an extremely common neurological disorder affecting around 300 million people worldwide. According to the World Health Organization (WHO). This disorder can also lead to idealizations of suicide which is a leading cause of death for individuals between 15 to 29. The disorder is characterized by behavioral symptoms of decreased mood, decreased activity, a change in appetite, and a change in sleep. In addition, major depressive disorder causes a decrease in levels of the neurotransmitter, serotonin, which is the primary neurotransmitter in depression. Currently, major depressive disorder relies on behavioral assessments to identify the disorder and little is known about its neurological biomarkers (Zhang, 2018). The disorder has been identified to affect many different lobes and areas of the brain, such as the frontal lobe, thalamus, hypothalamus, and parietal lobe.

The research on differences in symptoms, progression, and prevalence of many disorders and diseases between varying sexes and sexual orientations have become increasingly prevalent within recent years. This increasing amount of research has lent itself to emerging investigations on how symptoms may change between varying sexes and sexual orientations. There has been new research analyzing the difference of the biological makeup of males and females and it's been concluded that biological sex differs not only in the gonads but also within the entire body (Miguel-Aliaga, 2022). There are multiple different types of biological sex differences such as behavioral, chromosomal, and hormonal. But specifically, neurological sex differences have been found to be the primary cause of varying prevalence of neurological disorders between males and females, but also

between heterosexual and homosexual individuals. Between males and females, anatomical differences within the brain such as brain thickness, brain volumes, and surfaces have been defined as larger in males than in females (Ritchie et al.,, 2018). Between heterosexuals and homosexuals, anatomical differences of different lobes such as the hypothalamus and frontal lobe have on average been larger in heterosexual than homosexual individuals (Votinov et al., 2021). Due to this, neurotransmitter systems, specifically the ones of serotonin may potentially differ vastly. Current medicinal treatments of major depressive disorder consist of a major branch of antidepressants (Yuan et al., 2006). Some more popular antidepressants are selective serotonin reuptake inhibitors (SSRIs), serotonin-noradrenaline reuptake inhibitors (SNRIs), and mood stabilizers. SSRIs such as fluoxetine, sertraline, citalopram operate by blocking reuptake of serotonin and allowing for more serotonin to bind to the receptors and relaying more "happy" messages that serotonin is responsible for (Manninen et al., 2021). Mood stabilizers, most popularly lithium chloride, primarily operate by binding to serotonin receptors releasing a series of signals affecting neuroplasticity and neurogenesis ultimately affecting the symptoms of depression (Sangkuhl et al., 2009). These antidepressants affect the serotonergic systems of the brain which can lead to varying efficacies of treatment.

The model organism, *Drosophila melanogaster*, has been a popular model to study human behavior due to its similar genetic model and extensive gene homologs to humans. The *5HT* gene is a gene in humans that controls production and secretion of serotonin. It has many implications to mood disorders such as MDD and anxiety. Specifically, a mutation in the gene causes a disruption in the production of serotonin and serotonin levels are decreased causing depressive-like symptoms (Sangdee and Franz, 1980). The gene homolog is found in *Drosophila* and is a common gene used to model depression within fruit flies. The *fruitless* (*fru*) and *doublesex* (*dsx*) genes are heavily involved in the sex behavior of *Drosophila* where the *fru* gene mutation causes male-to-male courtship meaning the gene can be used to model homosexuality while not affecting female flies (Siwicki et al., 2009, Demir and Dickson, 2005). The *dsx* gene mutation can be used to model transgender behavior because it causes male flies to behave more like female flies with decreased courtship and females to behave more like males with increased levels of aggression. Although these are not perfect models for human behavior, these two genes also operate in the neuronal circuitry of *Drosophila melanogaster* affecting neurological behavior of the flies while not affecting the fly's migration towards food and have been used to model similar behaviors.

Depressive-like symptoms in *Drosophila* present themselves in various ways. Examples would include low food intake, a high preference for sugar, decreased movement, decreased sleep, and lower levels of serotonin. A higher preference for sugar and low appetite is seen in *Drosophila* affected with MDD (Ries et al., 2017). This is because individuals affected with MDD prefer to eat carbohydrates since carbs allow for an increase in serotonin and a boost in mood (Rao et al., 2008). Decreased movement and decreased sleep are highly characteristic of MDD in both humans as well as fruit flies and can be used to quantitate symptoms of depression (Yuan et al., 2006). Cyclic AMP (cAMP) is a common secondary messenger that is found in many pathways throughout the body. However, serotonin plays a role in the synthesis of cAMP which allows for cAMP to be a marker for serotonin levels (Lambert and Lauder, 1999).

In summary, the purpose of this investigation was to elucidate the effect of biological sex and sexual orientation on the response to different treatments of Major Depressive Disorder. This study includes a series of genetic crosses to be able to achieve dihybrid crosses as well as a trihybrid cross, exposing the groups of flies to the three different treatments, fluoxetine, sertraline, and lithium chloride, and then analyzing the way the symptoms can change across the previously mentioned groups. We hypothesized that if males, females, *fru* mutants, *dsx* mutants, 5HT1A mutants, and combinations of those received different treatments for MDD, then male flies will receive the most effective treatment with lithium chloride, female flies will receive the most effective treatment with fluoxetine, and *fru* mutant and *dsx* mutant flies will receive the most effective treatment with sertraline.

Materials + Methods

Genetic Crosses

The flies (Stock 25013, 26581, 24373 Bloomington Drosophila Stock Center) were kept at a 12hr light/dark cycle at 20ºC. The flies were kept on a diet of blue food. To cross the mutants, the flies with the gene mutation were combined together in a breeding chamber; when the flies hatched, the flies with the desired and identified phenotypes were isolated into a separate vial to ensure no loss of the gene mutation. After the F1 generation was created, the flies would continue to breed into future generations so all future generations would have the gene mutant cross wanted.

Name	FlyBase ID	Genotype	Mutant summary
5HT1A	FBgn0004168	Df(2R)BSC349	- mutation, decreased serotonin levels, models MDD
fru	FBgn0004652	Df(3R)BSC509	- mutation, models homosexuality
dsx	FBgn0000504	Df(3R)BSC729	- mutation, models transgender behavior

Treatments

The flies fed on 50mM lithium chloride (310468 from Sigma-Aldrich), 10mM fluoxetine (J61197.MF from ThermoFisher), and 0.82 mM sertraline (462190050 from ThermoFisher). To make the lithium chloride solution, 1.06g of lithium chloride should be dissolved in 500mL of water and that solution should be used to create the NutriAgar for the flies. To make the fluoxetine solution, 1.55g of fluoxetine should be dissolved in 500mL of water. To make the 0.82mM solution of sertraline, 125.55mg of sertraline should be dissolved in 500mL of water. Each group of flies were fed on the NutriAgar overnight.

Sucrose Preference Assay

A 2M solution of sucrose was prepared by dissolving 68.46g of sucrose into 100mL of distilled water. To prepare the preference mechanism, a cotton ball soaked with distilled water was placed into a 15mL centrifuge tube and a cotton ball soaked with the sucrose solution was placed into another 15mL centrifuge tube. Each centrifuge tube was attached to either side of a preference mechanism. Flies were added into the middle of the mechanism and left to prefer either side for a minute.

Excretion-Quantification Assay

An excretion-quantification tube was created which used a 50mL centrifuge tube. A 5mm hole was drilled into the airtight cap and small air holes were poked along the sides of the tube. A 20µL pipette tip filled with blue

food dyed with erioglaucine dye (861146 from Sigma-Aldrich) was added to the cap of the tube. 10 flies were added and the cap was sealed shut and the flies left to feed for 24 hours. After 24 hours, the flies and pipette tip were removed and the holes of the tube were taped shut. 5mL of distilled water were added and the tube was centrifuged to collect all excrement on the side. The absorbance of the dye was measured through a spectrophotometer at 630 nm and the values were compared to a standard curve.

DAM Locomotor Assay

The Drosophila Activity Monitor (DAM) system was used. Each system consisted of 32 holes to fit a 5mm glass tube with an adult fly in each. The system quantifies the amount of times the fly crosses the barrier within a time period, specifically 12 hours. To prepare the tubes, blue food is added to one side of the tube and each side is closed with a small piece of cotton after one adult fly is added into the tube. Each tube is placed into the activity system and the data is uploaded to the DAM computer software.

cAMP ELISA Assay

A cAMP ELISA assay kit was used to quantify the levels of serotonin within the fly body and brain (KGE002B from R&D Systems). A fly serum was prepared for every group by collecting ~20 flies and homogenizing the flies and adding 2mL of phosphate buffered saline and then centrifuging the content to create a supernatant and pellet. The pellet can be discarded as only the supernatant acts as the serum. Each serum was used as a sample and the instructions of the ELISA kit were carefully followed. The well plates absorbance densities were measured through a microplate reader at 540 nm and then the values were compared to a standard curve to determine the concentration of serotonin.

Results

Genetic Crosses

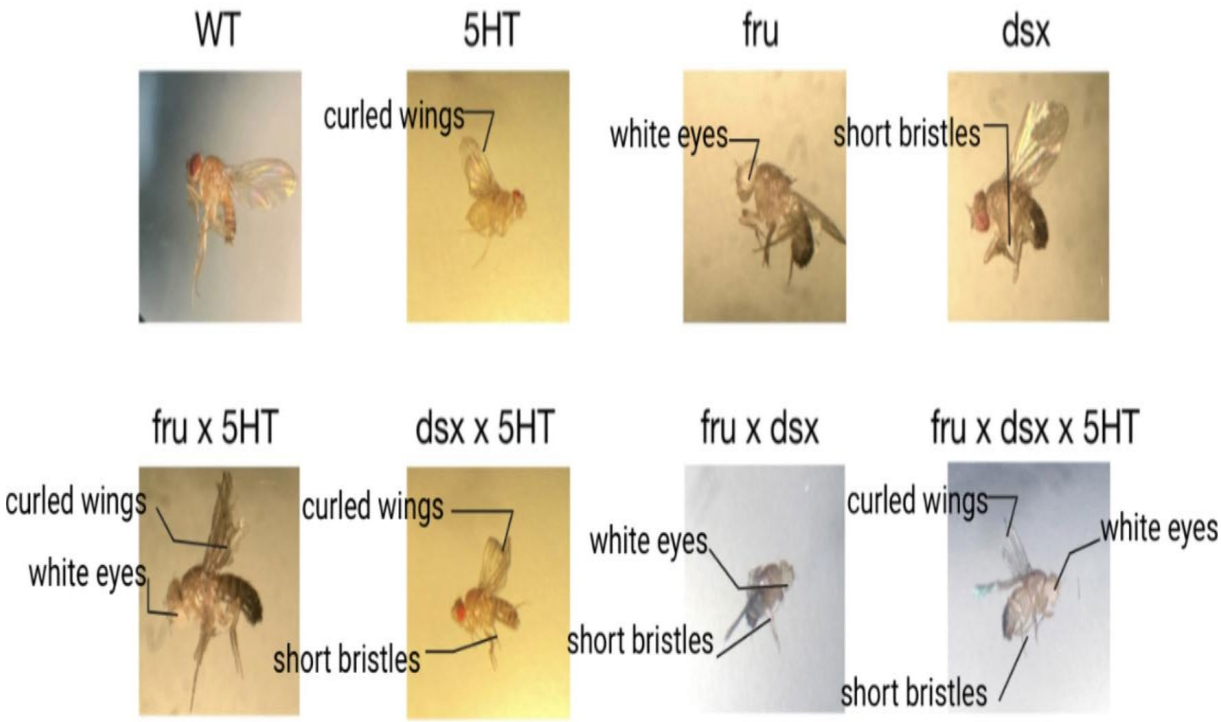

Figure 1. A collection of photos of mutant crosses. The fru mutant with a phenotype of white eyes, the dsx with short arm bristles, and 5HT with curled wings.

A series of different genetic crosses were done in order to create a *fru* x *dsx*, *fru* x 5HT1A, *dsx* x 5HT1A, and a *fru* x *dsx* x 5HT1A mutant. To identify if the flies had the gene mutation desired, balancer chromosomes producing a distinct phenotype were used. This caused the *fru* flies to have white eyes, the *dsx* flies to have shortened, yellow arm bristles, and the *5HT* flies have curled wings. The genetic crosses were all successful producing the phenotypes described previously (Figure 1).

Sucrose Preference Assay

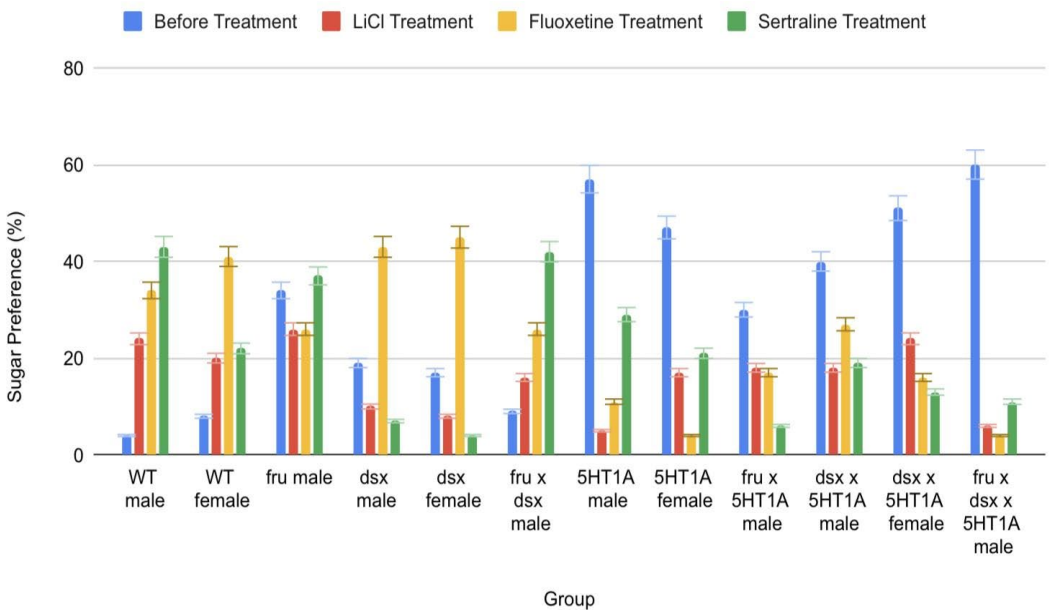

Figure 2. A sucrose preference assay shows the % of flies preferring sugar or water for all groups. An average of ten trials were utilized.

The sucrose preference assay uses a solution of sucrose on one side and distilled water on the other to see which the fly would prefer. Results showed that lithium chloride was most in WT males, fluoxetine in WT females, and sertraline most effective in *fru* and *dsx* mutants since those treatments decrease preference for sugar (Figure 2). Treatment groups without the *5HT* gene had either no response or an increased preference for sucrose (Figure 3, two-way ANOVA, $p<0.05$).

Excretion-Quantification Assay

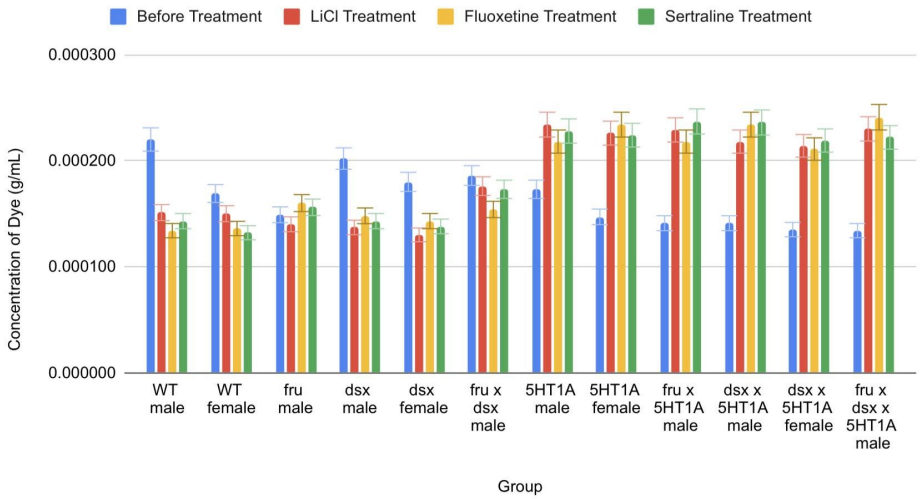

Figure 3. An excretion-quantification assay shows the % of flies preferring sugar or water for all groups. An average of eight trials were utilized.

The excretion-quantification assay tests the appetite of flies by dyeing the food, measuring the excrete through a spectrophotometer, and analyzing the absorbance of the dye through a standard curve. Lithium chloride was shown to be most effective in the male 5HT1A group at concentration, fluoxetine was most effective for the female 5HT1A group. For the *fru* x 5HT1A and *dsx* x 5HT1A groups, sertraline was most effective. The *fru* x *dsx* x *5HT* group had the most effective response with fluoxetine. This was seen since the treatments increased food intake within the respective groups (Figure 4). The non-*5HT* groups showed relatively similar numbers with no significant difference meaning the treatments had no effect on the food intake (Figure 5, two-way ANOVA, $p<0.05$).

DAM Locomotor Assay

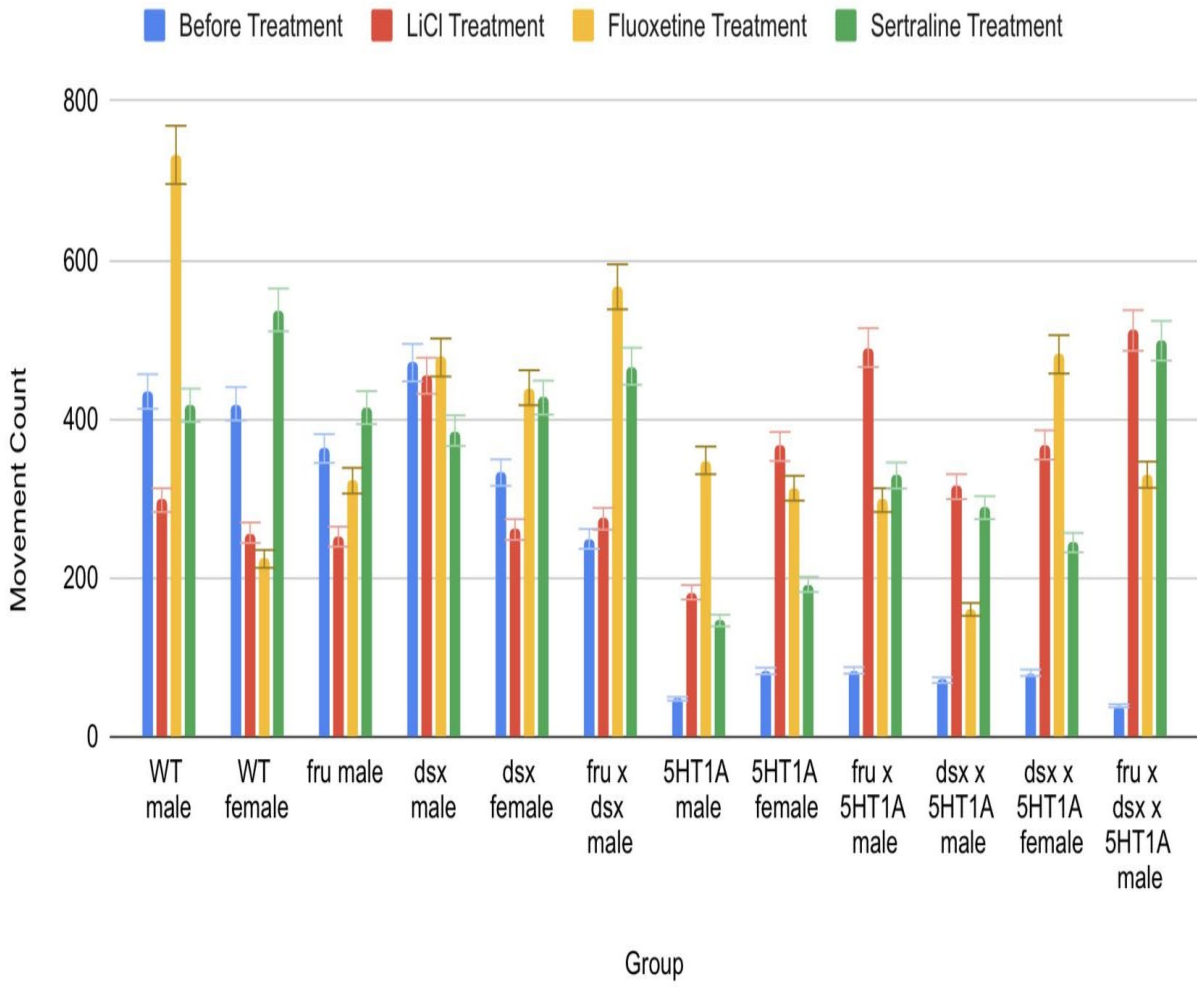

Figure 4. A DAM Locomotor assay shows the % of flies preferring sugar or water for all groups. An average of eight trials were utilized.

The DAM locomotor assay is done by placing a single fly in each glass 5mM tube and allowing the computer system to record how many times the fly crosses the barrier between each tube. Overall, lithium improved movement count at the highest rate for all groups except for the *5HT* males and the *fru* x *dsx* x *5HT* males where

fluoxetine had the highest improvement of movement (Figure 6). The non-*5HT* groups contained increased counts of movement (Figure 7, two-way ANOVA, $p<0.05$).

cAMP ELISA Assay

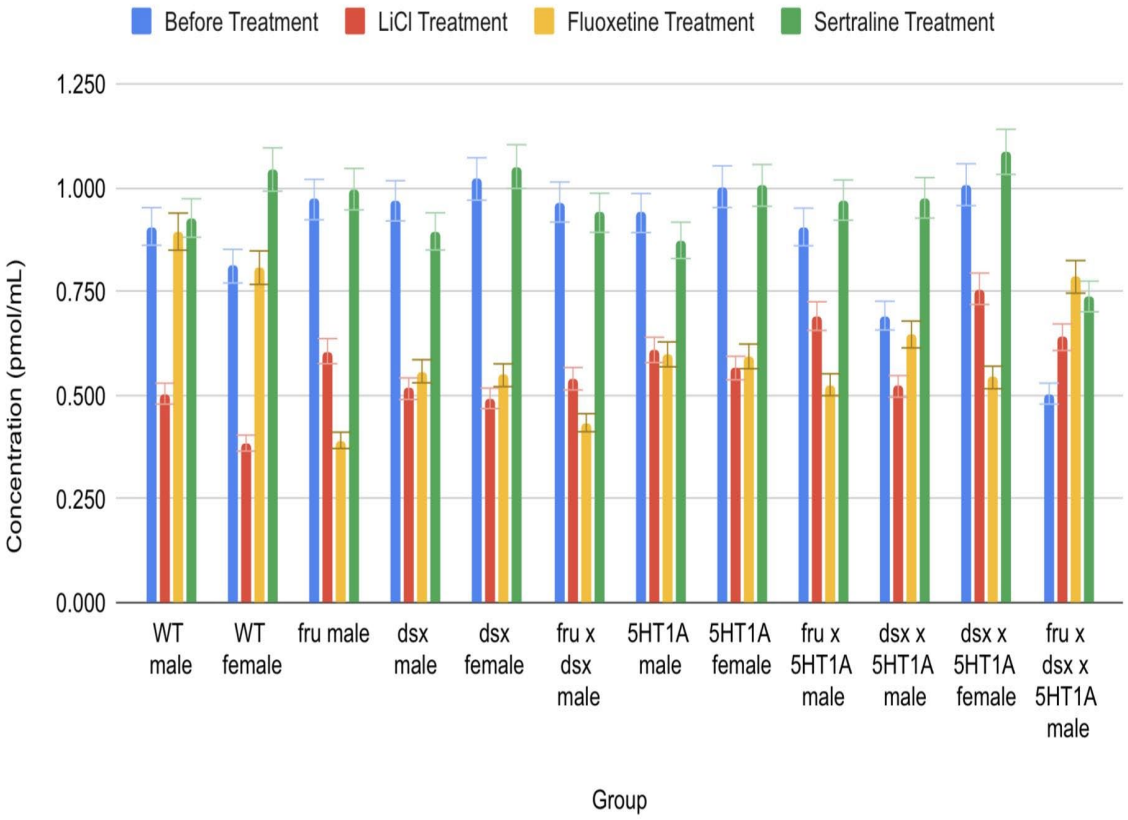

Figure 8. A cAMP ELISA assay shows the % of flies preferring sugar or water for all groups. An average of three trials were utilized.

The cAMP assay includes using an ELISA kit from R&D systems to monitor the serotonin levels within the fly brain and body. Overall, sertraline was most effective for all *5HT* groups and non-*5HT* groups except for the *fru* x *dsx* x *5HT* males where fluoxetine was most effective at raising sertraline levels (Figure 8 & 9, two-way ANOVA, $p<0.05$).

Discussion

Sucrose Preference Assay

The sucrose preference assay supported the hypothesis that lithium chloride would be most effective in males, fluoxetine for females, and sertraline for *fru* and *dsx* mutants. The treatments decreased the preference for sugar for their respectful groups. The control groups mostly had no response to the treatment and preference levels stayed the same.

Excretion-Quantification Assay

The hypothesis that lithium chloride would be most effective in males, fluoxetine for females, and sertraline for *fru* and *dsx* mutants was supported with the excretion quantification assay. Each group previously listed had an increase in food intake after treatment.

DAM Locomotor Assay

In the DAM locomotor assay, the hypothesis that lithium chloride would be most effective in males, fluoxetine for females, and sertraline for *fru* and *dsx* mutants was not supported. Lithium chloride was most effective for all groups instead of for individual groups having most effective treatments. This may be due to the mood regulating and increasing nature of lithium chloride.

cAMP ELISA Assay

In the cAMP ELISA assay, the hypothesis that lithium chloride would be most effective in males, fluoxetine for females, and sertraline for *fru* and *dsx* mutants was not supported. Sertraline was most effective across all the groups no matter the sex or sexual preference which could be due to the nature of sertraline where it is the most effective of the selective serotonin reuptake inhibitors.

Limitations

Limitations can include model organism restrictions, budget constraints, and issues with the technology utilized. *Drosophila melanogaster* are not the perfect model for human behavior as there can be limitations amongst homosexual and depressive behaviors between fruit flies and humans. In addition, the *fru* gene only acts upon male behavior and not female meaning female-to-female courtship preference was not able to be explored in this experiment. Furthermore, genetic crosses of the mutants could be affected through varying factors in the environment and possible incorporation of other gene mutations. Future research could consist of using a larger model organism such as mice or rats to be able to close the gap of differences between flies and humans and more closely model human behaviors through more accurate assays. Issues with technology and instruments to quantify various symptoms or absorbances can also be a limiting factor.

Applications

This scientific investigation can have many applications to the biomedical field of research and improve patient care. Major depressive disorder affects around 300 million people globally and can lead to death in certain cases. Antidepressants are not 100% effective and have an efficiency rate closer to 40-60%. Even so, antidepressants can often have adverse effects causing the symptoms of depression to worsen and maybe even increasing suicidal thoughts. With treatment of the disorder and many other diseases, drug trials have often only ever used male participants excluding a vast majority of other groups of people. This can cause the efficiency of therapeutic drugs to vary massively amongst non-males and most likely more efficiently within males. Improving the efficacy of treatment for the disorder is very important and highlighting the ways that these treatments can differ and identifying which treatment is more effective in which groups of people is imperative. Not only can this be applied to only major depressive disorder, but it can also be applied to somatic diseases such as heart disease or other neurological disorders such as ADHD. Overall, this can highly increase the rate of

symptom improvement of antidepressants, decrease drug dosage or amount of time that the patient has to be on medication, and also aid doctors in knowing which drugs to administer to patients.

In addition, lithium chloride was most effective for increasing activity and sertraline for serotonin levels implying that different therapeutic drugs can be more effective for certain symptoms. This could apply towards analyzing how different treatment applies to certain symptoms and improves patient care since certain patients may have an excess of a certain symptom and no signs of another symptom. Singular differences amongst groups such as one treatment being more effective with one symptom while another treatment being more effective with another symptom supports the claim that symptoms also have an effect on the sort of treatment best successful for the patient.

Future Research

Future investigation can use much more SSRIs such as paroxetine, escitalopram, and citalopram. Other different types of antidepressants such as serotonin noradrenaline reuptake inhibitors (SNRIs) or monoamine oxidase inhibitors (MAOIs) could also be explored. Furthermore, future research can include looking into non-neurological disorders like heart disease or diabetes.

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