The Effects of Prophylactic Fluoxetine Administration on Post-Stress Depressive-Like Behavior in Mice

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Depression is a devastating mental illness that is a leading cause of disability worldwide. Selective serotonin reuptake inhibitors (SSRIs) are the frontline pharmacotherapy, but at least a third of patients do not exhibit adequate treatment responses. While the causes of impaired treatment responses in humans remain unknown, prior work has shown that partial genetic inhibition of serotonin synthesis in mice impairs SSRI responses. This thesis aimed to determine whether low levels of brain serotonin impacted the effects of prophylactic fluoxetine administration on stress-induced alterations in depression- and anxiety-like behaviors in the light-dark emergence (LDE), elevated plus maze (EPM), sucrose splash test, and forced swim tests (FST). This experiment used tryptophan hydroxylase 2 (R439H) knock-in mice and their control wild-type (WT) littermates. Mice were split into groups based on drug treatment [fluoxetine (FLX) vs. water], stress status (stress vs. control), and sex (male vs. female). The results demonstrate that stress increased immobility in the FST and that FLX did not prevent this effect. In fact, in contrast to the hypotheses, FLX also reduced swimming behavior, although this effect was only significant in females. In the LDE, stress increased anxiety-like behavior in females but not males. In the EPM, most groups exhibited a stress-induced increase in anxiety-like behavior, but water-exposed WT males and FLX-exposed KI males exhibited significant reductions in anxiety-like behavior following stress. Overall, results suggest that females are more susceptible to stress than males and that oral fluoxetine is not an effective means to prevent adverse outcomes following stress.

Introduction

Depression is one of the most common mental illnesses worldwide. It is estimated that around 280 million people, or 3.8% of the worldwide population, suffer from depression (17). There are various front-line treatments for depression, including psychological approaches like talk therapy and pharmaceutical treatments like selective serotonin reuptake inhibitors (SSRIs). The use of psychotropic medications to treat depression has been steadily increasing over the past 40 years as pharmacological treatments are safer now than in the past (7, 9). The cause of depression is still up for debate, but the 5-hydroxytryptamine 5-HT (serotonin) deficiency hypothesis serves as a prominent explanation and area of study. According to this hypothesis, depression is caused by the dysregulation of brain serotonin. Earlier studies have found a connection between lower levels of 5-HT and its precursor L-tryptophan and individuals with major depressive disorder (MDD) (5). SSRIs increase extracellular levels of serotonin, which can improve neurotransmission and mood in a subset of individuals (5). While other neurotransmitters may contribute to MDD, SSRIs' efficacy and favorable side effect profile make them the most prescribed antidepressant medications for those with depression.

Previous research has shown that low-serotonin levels in mice have hindered both behavioral and therapeutic antidepressant methods both with and without stressors. Our experiment aims to uncover if providing antidepressants, like fluoxetine, prophylactically, will render different effects. Here, we are examining the effects of acute stress on wild-type and knock-in mice of both sexes, as research examining long-term or preventative effects of fluoxetine are limited. Our study aims to examine the effects of chronic prophylactic fluoxetine administration on post-stress behaviors as opposed to previous studies that have assessed post-stress behaviors following fluoxetine administration through injection, which can be an additional stress factor for the mice and is less generalizable to humans (6).

While SSRIs are commonly prescribed to treat depression, they typically have a 50% success rate (10). Patients who do not initially respond well to antidepressants are sometimes referred to as "treatment-resistant" or "treatment-refractory" (9). Treatment resistance for MDD is typically defined as a failure to respond or get relief from 2 or more medication trials of adequate dose and duration (20). One way in which healthcare providers have tried to combat this treatment resistance is by "switching, augmenting, and combining various pharmaceutical agents" (9). This switching, augmenting, or combining pharmaceutical agents includes prescribing medications across classes, such as SSRIs and tricyclic antidepressants (TCAs). Switching medications to treat major depression was successful in reducing symptomology 28% to 87% of

the time. These statistics do not account for a biological basis for SSRI treatment resistance in specific individuals (9). In cases of both switching and augmenting anti-depressant medications, some individuals experienced reduced symptomology. Researchers also pointed out that the rate of successful responses to a new medication decreases as the number of previous medication trials increases (9). While these data demonstrate that SSRIs and supplemental medications can be effective for some patients, further research regarding the biological bases for treatment responses and resistance has the potential to improve patient outcomes.

There are many potential reasons why an individual might not respond to SSRIs, including their prior exposure to other drugs, specific drug characteristics, or interactions with their other medications. More recently though, genetic variation has been proposed as another reason specific individuals might not respond to certain SSRIs or psychotropic medication more broadly (11). Evaluating the gene-drug interactions of specific individuals can lead to a greater understanding of their biological bases for treatment resistance. This evaluation of genetics in relation to medications relates to the field of pharmacogenomics, which studies the ways in which an individuals' genetic makeup may affect their response to drugs (1). Pharmacogenomics uses knowledge about slight differences in genetic makeups, the smallest differences being single nucleotide polymorphisms (SNPs), to predict how an individual might interact, both negatively and positively, with a certain drug (1). The difficulty of mapping individual genomes contributes to pharmaceutical companies' method of developing drugs on a one-size-fits-all basis; this strategy is based on how the 'average' person might respond. This method would serve as a possible explanation for the varying patient responses to SSRIs and their supplements or alternatives (1). Pharmacogenomics reveals how genetic composition could explain treatment resistance to antidepressants in humans.

While the exact causes of treatment resistance are generally unknown, there are several potential genetic mechanisms predicted to confer resistance to antidepressant treatment. One mechanism involves drug metabolism by the cytochrome p450 enzyme. Cytochrome p450 (CYP) are the major family of enzymes responsible for breaking drugs down in the body (18). CYPs can take on overly active or poor metabolizer forms, which can either result in a blockage of the metabolization of a drug or an over-active metabolization of a drug. This can lead to drug resistance or an increased risk of side effects, including overdose for some drugs (18). The poor-metabolizer polymorphisms of CYPs may cause adverse effects to drugs while ultra metabolizers may necessitate a higher dosage because of the speed at which the drug is cleared in the body (9). Individuals with ultra metabolizer CYP polymorphisms may not respond to SSRIs initially because of dosing issues.

CYPs also come in various forms, and their expression is highly dependent on individual genetic factors and other factors such as age, sex, and race (18). These differences in form and expression of CYPs in different individuals can impact the metabolization of drugs in the body and may serve as an explanation for resistance to anti-depressant medication. A second mechanism involves potential mutations in the primary pharmacological target of SSRIs, the serotonin transporter (SERT). Indeed, studies have found that genetic variants in serotonin transporters are associated with treatment responses to SSRIs. In studies done in humans, researchers have found a connection between serotonin transporter expression and drug response. They found that the expression of certain alleles in the SERT can result in non-response, suppressed response, or delayed response of SSRIs (15).

Third, SSRIs work by enhancing the extracellular levels of endogenously produced 5-HT. In the absence of 5-HT production, blocking the reuptake of 5-HT would not be predicted to enhance extracellular levels of 5-HT. Indeed, studies have shown that mutations in the 5-HT-synthesis enzyme, tryptophan hydroxylase 2 (Tph2), have been associated with antidepressant treatment responses. Genetic studies in humans are by default correlational, as it would be both unethical and unfeasible to make genetic manipulations in patients to evaluate their potentially harmful effects. Consequently, genetically modified mice are often used to determine the impact of particular genetic mutations on sensitivity to drugs or susceptibility to stress. Mutations or loss of function of the (Tph2) enzyme in the brain impacts 5-HT function. Genetically modified mice expressing the R439H mutation in Tph2 exhibit behavioral abnormalities and less production of 5-HT in the brain (4). This specific genetic mutation is important to this study because mutation of the Tph2 enzyme was also found in several people within a sample of individuals with MDD, suggesting that this enzyme mutation may be connected to an MDD diagnosis (19). Mutations of the Tph2 enzyme in the brain and 5-HT deficiency have been shown to block or hinder antidepressant treatment methods in mice, both psychotropic (i.e., pharmaceuticals) and behavioral (i.e., exercise) (2). Further, researchers also found a correlation between pharmaceutical treatment resistance and depression severity in individuals (2). In the same way that some individuals are resistant to SSRIs for treatment of their depression, experimental low-serotonin mice were found to show resistance to chronic administration of fluoxetine, as the medication did not cause antidepressant-like effects (13). In another study examining the effects of exercise in Tph2 knock-in (KI) mice, 5-HT deficient mice who underwent an exercise paradigm showed less antidepressant behaviors than control mice (16).

Where there is a correlation between low-

serotonin levels and mutation in the Tph2 enzyme, there may also be an increase in stress susceptibility. In addition to having diminished responses to antidepressants, Tph2R439H (KI) mice have been reported to have higher stress susceptibility to certain stressors, such as social defeat, when tested at stress levels insufficient to induce behavioral disturbances in wild-type (WT) mice (14). Tph2KI animals and WT mice have shown similar behavioral responses to full-intensity social defeat, but Tph2KI animals show increased vulnerability to sub-threshold, shorter-duration social defeat stress (14). These mice have also shown similar behavioral responses to WT mice to full-intensity chronic mild stress paradigms, but it has not yet been established if Tph2 mice would show similar behavioral responses to shorter durations of mild stress exposure. Prior work using mice that are not genetically modified has shown that a five-day stress paradigm including two days of restraint, two days of forced swim, and a day of tail suspension leads to significant alterations in behavior across a range of depression- and anxiety-like tests (3). As low serotonin levels caused by Tph2 enzyme mutations have hindered both behavioral and therapeutic antidepressant methods both with and without stressors, researchers have attempted to use antidepressants therapeutically to reverse the effects of stress in mice. As previous research indicates, 5-HT deficient mice were more susceptible to stress, but also the deficiency hindered the therapeutic-like effects of fluoxetine both in the presence (14) and absence of stress (13). Studies have looked at the efficacy of prophylactic fluoxetine administration in humans with major depressive disorders as fluoxetine is not only effective as an acute treatment but may also provide relief in the long term (12). Earlier studies in humans focused on longitudinal studies and yearly follow-ups to ongoing fluoxetine for treatment of MDD and treat or continue to treat individuals who have already expressed signs of MDD.

Depression studies including both male and female models are limited. As studies have shown that depression and anxiety may manifest differently in males and females due to differences in neural pathways and hormonal systems (8), it is important to consider that both male and female mice were included in our experiment. Previous research studying sex differences in response to subchronic variable stress found that more genes were regulated in the nucleus accumbens in stressed males than unstressed males and female mice (8). Further, there were behavioral differences in the responses to stress between males and females. While males appeared more resilient to stressors, females were more susceptible, exhibiting more depression-like behaviors (8). While researchers indicated that the interpretation of the behavioral differences between males and females needs further research, understanding that differences can occur gives insight into detection and treatment of depression in humans.

We predict that stress will impact both wild type and Tph2KI mice with female and low- serotonin mice displaying more susceptibility to stress than males and wild type mice. Further, we predict that stress will increase anxiety-like and depressive-like behaviors in mice, but more so in female and low-serotonin mice. Based on past research suggesting that low-serotonin based on mutations of the Tph2 enzyme can block the effects of antidepressants, we predict that chronic prophylactic exposure to fluoxetine will display preventative effects in the WT mice but not in the KI mice.

Methods

In this experiment, eight experiment groups were broken down into four staggered cohorts for experimentation—cohorts 1 (N=24), 2 (N=22), 3 (N=19), and 4 (N=20) (Cohort 2 began with 23 mice but ended with 22 mice as one mouse needed to be removed from the cage due to aggression in the home cage)—meaning experimentation for each cohort was performed at separate times. The mice were from the tryptophan hydroxylase 2 R439H knock-in mouse line and experimental animals were homozygous for either the wild-type (WT) or Knock-In (KI) allele (4). Mice were housed in identical clear cages by cohort with two to five mice per cage (Figure 1). Experimental mice were sometimes housed with heterozygous mice not used for experimentation to avoid singly housing mice. Cohorts 1 and 3 consisted of male mice, while mice in cohorts 2 and 4 were female.

Figure 1. ***Housing.*** *Mice were housed in cages ranging from 2 to 5 mice per cage. Mice were separated by sex, and no mouse was singly housed.*

Fluoxetine Administration

Fluoxetine (FLX) capsules of 20mg or 40mg were dissolved in either 133.3mL or 266.66mL, respectively, of standard drinking water to make a fluoxetine-water solution

with a final concentration of 150mg/L. Each bottle was weighed before placing it in its respective cage. Every three days, the bottles were removed from the cages, weighed, and emptied. The bottles were refilled with 45mL of the fluoxetine solution, weighed , and put back in cages. The fluoxetine regimen began 21 days prior to five-day-stress and was maintained throughout stressing and testing (note: Cohort 3 received 14 days of the fluoxetine solution before five-day- stressing began). The weights of the bottles were recorded to track an estimate of how much fluoxetine each mouse consumed. Additionally, each mouse was weighed each week, and weights were recorded. This was done to track any weight changes that might have occurred due to the fluoxetine.

Five-Day Stress

The five-day stress (5DS) paradigm was conducted as previously described but altered slightly for this experiment (3). This experiment did not include novelty suppressed feeding (NSF) or a sucrose preference test (SPT).

Restraint Stressor

For this procedure, on day 1, each mouse was put in a ventilated plastic 50mL conical tube for an hour (Figure 2). There were three Cohort 1 mice that were too big for the standard conical tubes used for this experiment, so larger tubes were made from a small section of PVC pipe. Mice were left in the tubes for an hour, and experimenters checked them consistently throughout the experiment. The food and water of the control mice who were not placed in the conical tubes was withheld for that same hour to keep the lack of access to food and water consistent. After the hour, the mice were removed from the tubes, and the water and food were returned to all the mice.

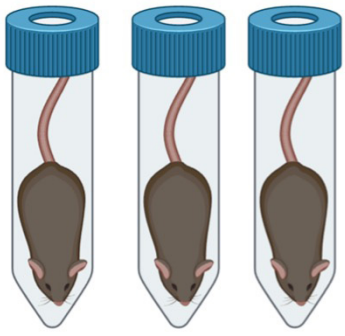

Figure 2. ***Restraint stressor.*** *Mice are placed in ventilated, conical tubes for an hour.*

Forced Swim Stressor

On Day 2, 4000mL beakers were filled with 2500 mL of room temperature water (27 degrees Celsius). Mice were carefully placed on the surface of the water, to prevent dipping their heads below the surface of the water (Figure 3). Mice were left in the water for 12 minutes under careful surveillance to ensure they stayed afloat. In the case that a mouse appeared unable to keep its head above water, it was removed from the beaker, dried off thoroughly with a paper towel and placed back in the cage and atop a heating pad to re-stabilize. After 12 minutes, mice were removed from the beakers by their tails, dried off with paper towels, and placed back in their cages.

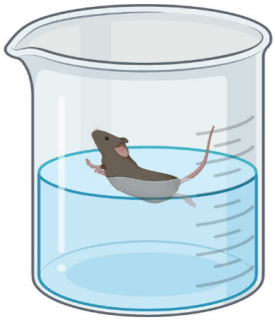

Figure 3. ***Forced swim stressor.*** *Mice are placed on the surface of the water in a 4000mL beaker filled with 2500mL of water for 12 minutes.*

Tail Suspension Stressor

On Day 3, mice were suspended by their tails for an hour under surveillance (Figure 4). Experimenters checked the mice every 10 minutes to ensure they did not climb to the top of the wood from which they were suspended. If the mice were no longer suspended, experimenters carefully used a pen to return the mouse to a suspended position. Similar to the restraint procedure, food and water from control cages were removed for the duration of the hour and returned at the end of the suspension period. When the hour was up, mice were taken down, the tape was carefully cut off their tails, and they were placed back in their cages.

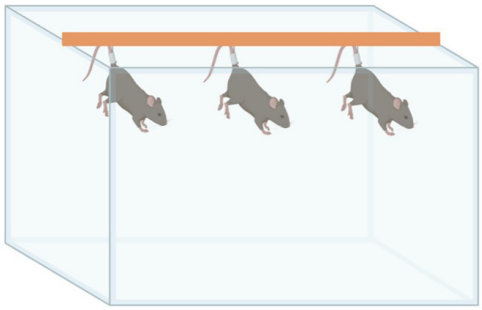

Figure 4. ***Tail suspension stressor.*** *Model of the tail suspension procedure. Mice are suspended from a plank of wood atop an open-field box by a piece of tape on their tails.*

Day 4 of the 5DS repeated the restraint procedure from Day 1. Day 5 of the 5DS repeated the forced swim procedure from Day 2.

Behavioral Testing

After the 5DS paradigm was completed, a four-day behavioral testing period began. Four-day testing was conducted as previously described but altered for this experiment (3). This experiment followed Baugher's procedures for light-dark emergence, sucrose splash test, elevated-plus maze and forced swim behavioral testing protocol but did not include novelty suppressed feeding or sucrose preference test protocol (3).

Light Dark Emergence

On Day 1, a light-dark emergence (LDE) test was conducted. LDE testing was performed using the ANYBox system. Acrylic boxes were placed on the lab bench under their respective cameras that connect to the ANYMaze system on the computer. One side of the box is clear, allowing light through and the other is blacked out and covered, not allowing light in. To begin the testing, each mouse was placed in the dark side of the light-dark emergence box, and the testing timer was started (Figure 5). Testing ran for five minutes, with ANYMaze tracking the number of times the mouse ventured to the light side of the box and how long it spent there.

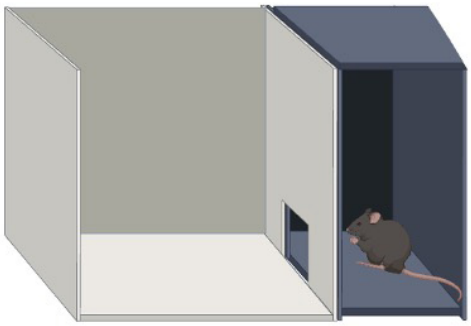

Figure 5. ***Light-dark emergence testing.*** *Light-Dark Emergence Box*

Sucrose Splash Test for Grooming Behavior

On Day 2, a splash test was conducted. A clean, new, empty cage was set up on a tabletop in front of a plain, white background, and a camera was set up in front of the cage (Figure 6). A 10% sucrose solution was prepared and put in a spray bottle. To begin testing, a mouse was placed in the cage. Upon beginning the camera recording and a 5-minute timer, , the mouse ID was shown to the camera, and an experimenter sprayed the mouse in the cage three times with the sucrose solution. The experimenter then covered the cage, left the mouse, and avoided interfering with the recording. After five minutes, the recording was stopped, and the mouse was removed from the cage. The cage was then reset for the next mouse, which included being sprayed down to rid the cage of sticky residue from the previous mouse. This process was repeated for all mice.

Figure 6. ***Sucrose splash test for grooming behavior.*** *Splash test set-up. A mouse is placed in a clean, empty cage in front of a neutral background in front of a camera. The mouse is sprayed with a 10% sucrose solution and left for 5 minutes.*

Elevated Plus Maze

On Day 3 of behavioral testing, an elevated plus maze (EPM) test was run. A structure with four arms, two enclosed and two open, was set up in the lab below a camera connected to the ANYMaze system on the computer. One by one, mice were placed in one of the closed arms for 5 minutes (Figure 7). ANYMaze tracked the location and movement of the mouse to determine if they ventured to the open arms and for how long.

During this process, one of the mice fell off the elevated plus maze. After being retrieved, the mouse was returned to the maze and testing continued. All mice were tested. The EPM measured total distance, open arm: entries, open arms: head entries, open arms: time(s), open arms: distance (m), open arms: latency to first entry (s).

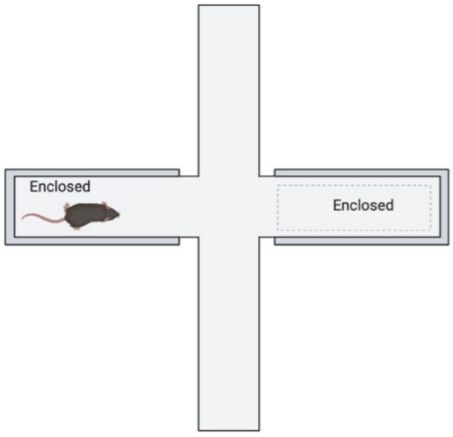

Figure 7. ***Elevated plus maze testing.*** *Elevated Plus Maze. A mouse is placed on one of the two enclosed arms, and its movement is tracked using ANY maze to determine if the mouse ventures to the open arms.*

Forced Swim Testing

On Day 4 of behavioral testing, a forced swim behavioral (FST) test was run. Similar to the forced-swim stressor, mice were placed on the surface of the water in a 4000 mL beaker filled with 2500mL of water (Figure 8). For behavioral testing, each beaker was placed under a camera connected to the ANY Maze system on the computer. The system tracked the amount of time the mice swam and how long they were immobile. In the case that a mouse appeared to dip its head or sink below the water, it was removed from the beaker, dried off thoroughly with paper towel and placed back in the cage and onto a heating pad to re-stabilize. In some cases, in cohort 4, mice had to be removed from behavioral testing immediately as they were unable to maintain their heads above the water. Testing was stopped and this data was not used for analysis purposes. These mice were taken out, dried off, and placed in their cage on a heating pad to re-stabilize, as previously stated in the stressor protocol. After the five minutes of testing was completed, mice were removed from the beakers by their tails, dried off with paper towel, and placed back in their cages.

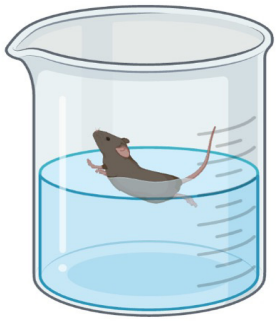

Figure 8. ***Forced swim test behavioral testing.*** *Mice are placed on the surface of the water in a 4000mL beaker filled with 2500mL of water for 12 minutes.*

Behavioral Testing Measures

The LDE measured light: entries, light: time (s), light: distance (m), and light: latency to first entry (s). The EPM measured the total distance traveled (m), entries into open arms, head entries to open arms, time in open arms (s), distance in open arms (m), and latency to first entry to open arms (s). The splash test was scored manually and measured the amount of time spent grooming (s) and grooming bouts. The forced swim test measured distance (m), time immobile (s), immobile episodes, and immobility latency (s).

Statistical Analyses

For statistical analysis, SPSS was used to conduct a 4-way ANOVA (2x2x2x2) as each of the conditions of drug, stress, genotype, and sex had two levels: fluoxetine group/water group, stress/no stress, KI/WT, and male/female. When significant interactions were observed, individual group differences were assessed via post-hoc testing using Bonferroni tests. P values below 0.05 were statistically significant.

Results

Statistical analysis for the FST (Figure 9) shows that stress mainly impacted total distance ($F(1,82)=170.683$, $p<0.001$), time immobile ($F(1,82)=45.474$, $p<0.001$), immobile episodes ($F(1,82)=36.950$, $p<0.001$), and immobility latency ($F(1,82)=121.052$, $p<0.001$). Control stress mice swam more distance than stressed mice. Stress-exposed mice spent more time immobile and had more immobile episodes. Control stress mice had a higher immobility latency time than stressed mice. Analyses also show that there was a main effect of genotype on total distance ($F(1,82)=21.061$, $p<0.001$), time immobile ($F(1,82)=14.620$, $p<0.001$), and immobile episodes ($F(1,82)=15.760$, $p<0.001$). KI mice swam more distance than WT mice. WT mice spent more time immobile and had more immobile episodes. There was also a main effect of drug on total distance ($F(1,82)=21.981$, $p<0.001$) and time immobile ($F(1,82)=7.390$, $p=0.008$) in which control FLX mice swam more distance than FLW mice and FLW mice spent more time immobile than control FLX mice. Lastly, there were main effects of sex on total distance ($F(1,82)=21.773$, $p<0.001$), time immobile ($F(1,82)=38$, $p<0.001$), and immobile episodes ($F(1,82=8.992$, $p=0.004$). Female mice swam more than male mice. Males spent more time immobile and had more immobile episodes. Analyses also reveal that there were significant interactions. There was a drug by sex interaction on total distance ($F(1,82)=8.182$, $p=0.006$) and immobile episodes ($F(1,82)=7.372$, $p=0.008$) as there were significant differences in the ways in which drug groups responded based on sex. In both total distance and time immobile, we see similar trends in the male and female groups but at varying magnitudes. We also found a four-way interaction of sex by genotype by drug by sex on time immobile ($F(1,82)=6.072$, $p=0.016$).

Light-dark emergence analyses (Figure 10) show that there was a main effect of genotype on light entries ($F(1,84)=8.385$, $p=0.005$) in which WT mice had more entries into the light chamber than KI mice. There were also main effects of drug on distance traveled in light chamber ($F(1,84)=4.078$, $p=0.047$) and latency to first entry into the light chamber ($F(1,84)=4.485$, $p=0.031$). The water group spent more time in the light chamber. The FLX mice had a longer latency to first entry into the light chamber. There were main effects of stress on time spent in the light chamber ($F(1,84)=14.673$, $p<0.001$), distance traveled in the light

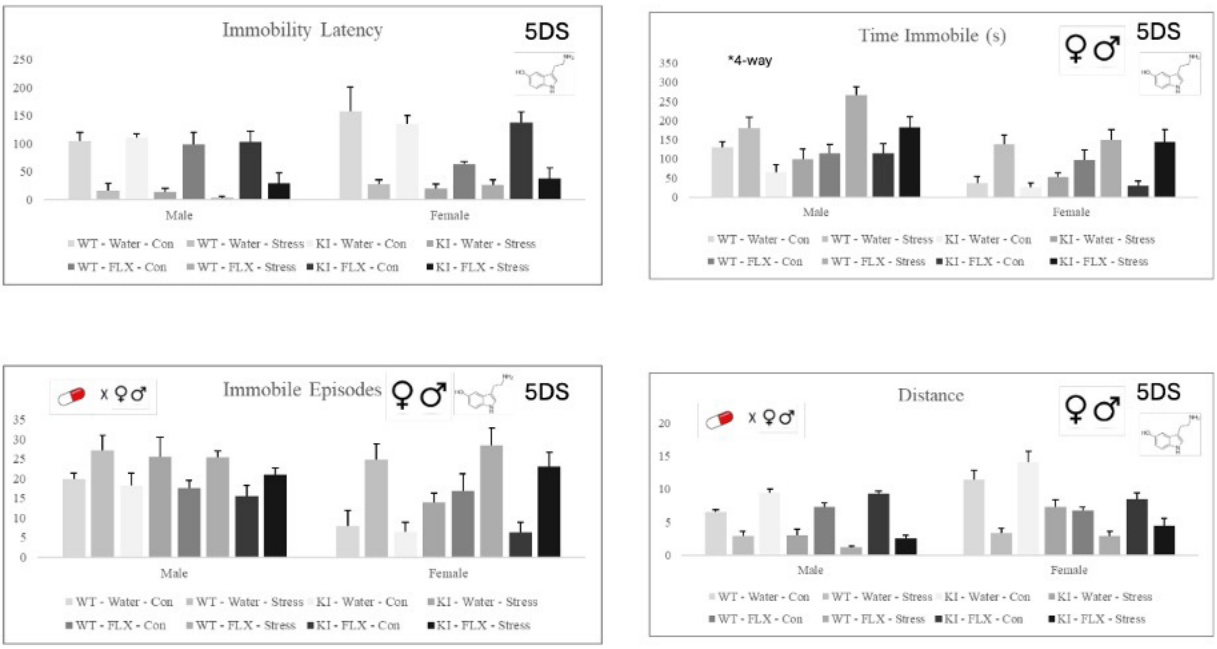

Figure 9. ***Forced swim test data.*** *FST data shows that there was a main effect of stress on all measures, a main effect of genotype on total distance, time immobile, and immobile episodes, and a main effect of drug on total distance and time immobile. There was a drug by sex interaction on total distance and immobile episodes. There was a 4-way interaction on time immobile.*

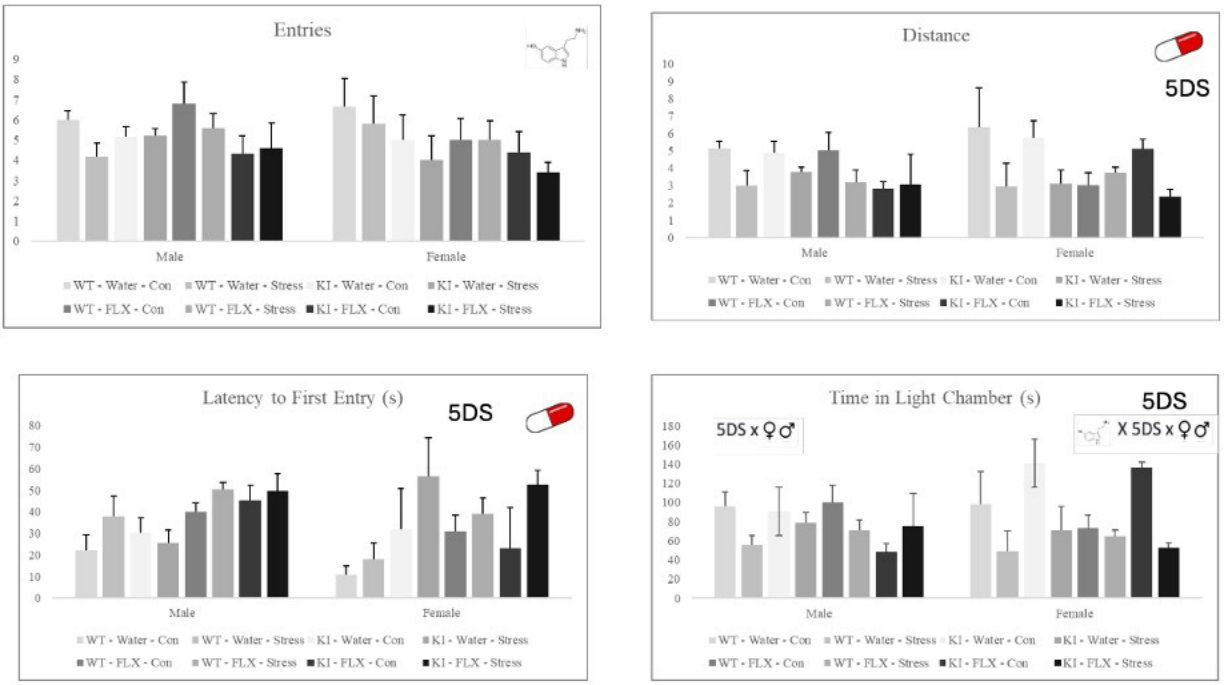

Figure 10. ***Light dark emergence data.*** *Light-dark emergence data shows a main effect of genotype on light entries, a main effect of drug on light distance and latency to first entry, and a main effect of stress on light time, light distance, and latency to first entry. Results also show a stress by sex interaction on light time and a 3-way genotype by stress by sex interaction on light time.*

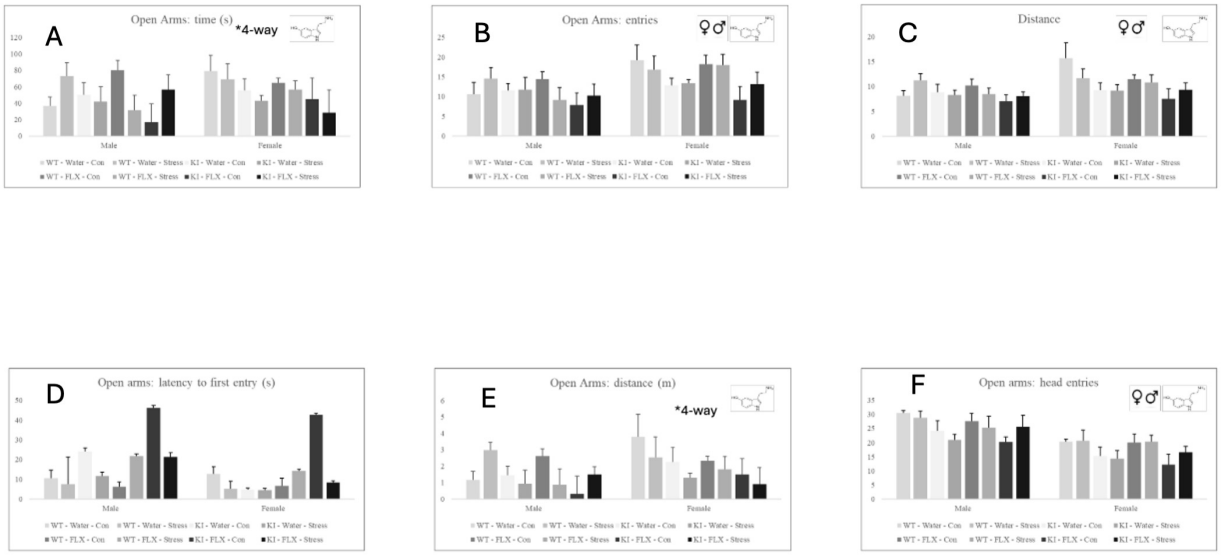

Figure 11. ***Elevated plus data.*** *EPM data shows a main effect of genotype on total distance, open arm entries, open head entries, open arms time, and open arms distance. There is a main effect of sex on total distance, open arm entries, and open arms head entries. There is also a 4-way interaction in open arms time and open arm distance.*

chamber (F(1,84)=12.600, p<0.001), and the latency to first entry (F(1,84)=5.242,p 0.025). Control mice traveled more distance and spent more time in the light chamber than stressed mice Stress-exposed mice had a higher latency to first entry than control mice. We also found a two-way stress by sex interaction on time spent in light chamber (F(1,84)=5.689, p=0.020) and a three-way genotype by stress by sex interaction on time spent in light chamber (F(1,84) = 4.712, p=0.033). In this genotype by stress by sex interaction, the females demonstrate significant differences in time in the light chamber between control and stress groups, where control groups spent more time in the light chamber than the stress groups. This was true in all cases except for the WT-FLX group, in which there was not a significant difference in time spent in the light chamber between the control and stress group.

EPM (Figure 11) revealed main effects of genotype on total distance (F(1,85)=13.278, p<0.001) (Figure 11.C), entries to open arms (F(1,85)=7.534, p=0.008) (Figure 11.B), open arms head entries (F(1,85)=14.577, p<0.001) (Figure 11.F), time spent in open arms (F(1,85)=6.303, p=0.014) (Figure 11.A), and distance traveled in open arms (F(1,85)=8.039, p=0.006) (Figure 11.E). WT mice traveled more distance overall, entered the open arms more frequently, put their heads into the open arms more frequently, spent more time in the open arms overall, and traveled more distance in the open arms than the KI mice. We also found main effects of sex on total distance traveled (F(1,85) =7.102, p=0.010) (Figure 11.C), entries to open arms (F(1.85) =7.340, p=0.008) (Figure 11.B), and head entries to open arms (F(1,85) =30.091, p<0.001) (Figure 11.F). Females traveled more distance overall and entered the open arms more frequently than males. Male mice put their heads into the open arms more frequently than females. We also found a four-way genotype by drug by stress by sex interaction for open time (F(1,85) =5.264, p=0.025) (Figure 11.A) and open distance (F(1,85) =4.014, p=0.049) (Figure 11.E). The genotype by drug by stress by sex interaction suggests that the magnitude of these differences in sex and genotype depended on the stress and drug conditions of the mice.

Splash test analyses (Figure 12) revealed main effects of stress on grooming time (F(1,85) =8.053, p=0.006). Control mice spent more time grooming than stress mice. There was also a main effect of drug on grooming time (F(1,85)=6.519, p=0.013) in which the water group spent more time grooming than the FLX group. There was a main effect of sex on grooming time (F(1,85) =11.734, p=0.001) and grooming bouts (F(1,85) =4.925, p=0.030). Female mice spent more time grooming than males and had more grooming bouts than males. There were no significant interactions.

Discussion

Our hypotheses were only partially supported by our results. Splash test results showed no significant interactions. As expected, control mice displayed more grooming behavior than stressed mice and spent more time grooming. Overall, prophylactic fluoxetine administration in the drinking water was not an effective preventative measure for depressive and anxiety-like behaviors in mice. Although one could argue the lack of a significant difference

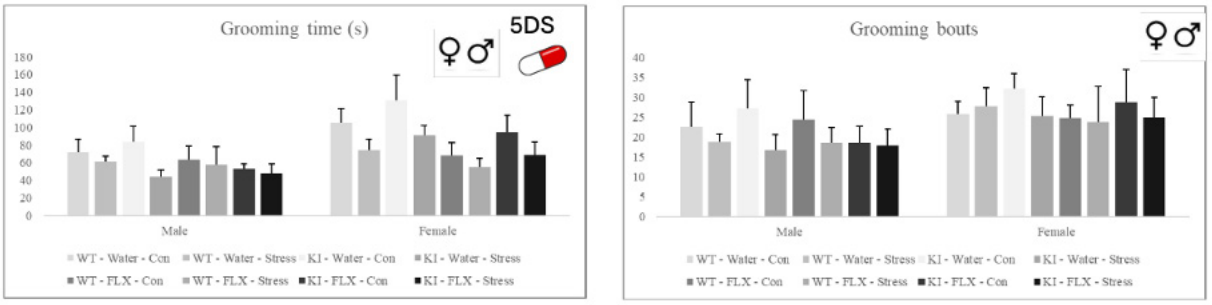

Figure 12. ***Sucrose splash test data.*** *Splash test data reveals that there is a main effect of stress on grooming time, a main effect of drug on grooming time, and a main effect of sex on grooming time. There was also a main effect of sex on grooming bouts. There were no significant interactions.*

between the stress-exposed WT FLX female group and the control WT FLX female group could reflects protection against the effects of stress, FLX appeared to induce a pro-anxiety phenotype like the effect of stress. So, this does not appear to be beneficial; LDE light time results also suggest that fluoxetine had a detrimental effect on KI males and neither helped nor hurt KI females in either stress or control groups. The FST reveals that stress increased immobility overall, which was expected. However, we also hypothesized that fluoxetine might not assist the KI group but would reduce immobility in the WT group. WT mice females did not experience positive effects from fluoxetine administration, but KI females showed detrimental effects. While this does not entirely align with our hypothesis, it aligns in the sense that we initially thought WT mice would respond more positively to fluoxetine than KI mice. Here, having no response to fluoxetine is a positive reaction compared to the detrimental reaction we see in KI female mice.

EPM data shows that female mice were more active overall in testing and WT mice were less anxious overall. Neither stress exposure nor FLX exposure had a significant impact on behaviors in the EPM, but there were sex and genotype differences observed. The genotype by drug by stress by sex interaction suggests that the magnitude of these differences in sex and genotype depended on the stress and drug conditions of the mice. While fluoxetine administration did not show preventative or therapeutic effects in these tests, KI stress females exhibited somewhat of an adverse response to FLX as female KI-FLX stressed mice spent significantly less time in the open arms than their male counterparts.

This study was conducted to gain insight into the connection between genetics, mental illness, and pharmacotherapy. With an ever-evolving pharmaceutical industry, deeper understanding of drug mechanisms is necessary for improving access and treatment for individuals. Further, gaining insight on treatment-resistance in individuals gives insight into the successes and places for improvement within the pharmaceutical industry. Individual genetic differences that can result in different metabolisms for different drugs suggests that the on-size- fits all approach of the pharmaceutical industry is not sustainable. Further, learning about differences in response to stress and drugs based on genotype and sex is imperative in gaining deeper understanding into the prevalence rates for diagnoses and prognoses.

Studying the effects of prophylactic fluoxetine administration following chronic stress also gives insight into the function of fluoxetine in the varying behavioral manifestations of stress in both low-serotonin and normal instances. By examining the preventative effects of fluoxetine consumption, we can gain insight into the function or importance of consistent SSRI use between depressive episodes instead of acute use as needed. As earlier studies have shown, long-term SSRI administration after depressive symptoms can be effective in preventing future episodes. Our research aims to expand upon this by examining the effects of fluoxetine on preventing adverse behaviors to chronic stress and examining factors of genetic difference and sex on these effects as well.

Limitations

There were some minor setbacks in the research that did not significantly affect results but must be noted. While cohorts 1, 2 and 4 were given fluoxetine 21-days prior to first stress exposure, cohort 3 was given fluoxetine for 14-days due to a scheduling error. Statistics were independently run for cohort 3 and showed similar trends to the other three cohorts and thus were included in the overall statistics.

While statistics were not gathered cage by cage, it must be noted that mice were housed in cages varying from two to five mice. While there were never more than four male mice in a cage or more than five female mice in a cage, it is unclear whether the number of mice in each cage impacted the behaviors of the mice. While singly housing mice is avoided to prevent stress caused by lack of social interaction, it is unclear if the number of mice per cage impacted behavior.

Additionally, there were some instances where

specific data could not be used because of difficulties with the mice. For example, the LDE behavioral testing demonstrated some technical difficulties tracking mouse location, so F values vary for that test. Additionally, some trials in the forced swim test had to be omitted as some of the mice began struggling in the water. Statistical analyses revealed that these omissions did not impact the overall outcomes.

Aside from experimental obstacles and limitations, generalization and application of the results is not entirely possible as mice were exposed to an acute 5-day-stress paradigm and assessed for adverse behaviors immediately afterward while still on fluoxetine. While this experiment can explore the preventative abilities of fluoxetine, long-term effects cannot be concluded. Though mice were exposed to a chronic fluoxetine regimen for 21-days prior to stress and throughout stressing and testing, the effects of fluoxetine in the interim between stressful events or depressive episodes cannot be determined. Further, earlier research indicates that age and previous exposure to stress and drugs can impact the serotonin pathways. Experimenting with mice of different ages and controlling for age might yield different results. Also experimenting with a different stress and drug schedule might allow for more generalizable results. Further, as it has been established that males and females respond to stress differently, an experimental model centered around sex differences and treatment could provide more insight into treatment methods. To gain more insight into the purpose of chronic fluoxetine administration for people with MDD and the preventative abilities of fluoxetine between depressive episodes, it could be beneficial to put mice through multiple rounds of 5DS with chronic fluoxetine exposure and assess the role of fluoxetine over a longer period of time. More research into chronic fluoxetine and stress is needed to fully understand its effects.

Conclusions

Chronic fluoxetine administration in drinking water was not an effective preventative measure against the effects of stress in either KI or WT mice, but we did see dysfunction caused by stress in most behavioral tests. While females did display increased stress susceptibility in the LDE, serotonin deficiency did not impact stress susceptibility. Continuing to research genotypic differences in 5-HT synthesis, the metabolism of SSRIs, sex differences in stress susceptibility and drug metabolism, and the roles of SSRIs in preventative and remedial treatments for mental illness is imperative in building a more comprehensive understanding on pharmacotherapy for mental illness.

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