Effects of Fluoxetine in Mitigating Depressive Symptoms in Socially Isolated Zebra Finches

by

Yuwei Liu, Michael Cory Mehlman & Allyssa Michelle Sullivan

A thesis submitted in partial fulfillment of the requirements
for graduation with Honors in Psychology.

Whitman College
2020

Certificate of Approval

This is to certify that the accompanying thesis by Yuwei Liu, Michael Cory Mehlman & Allyssa Michelle Sullivan has been accepted in partial fulfillment of the requirements for graduation with Honors in Psychology.

____
Nancy Day

Whitman College
May 20, 2020

Table of Contents

List of Figures

Acknowledgements

First and foremost, we would like to thank our thesis advisor, Professor Nancy Day, for her continuous motivation, perseverance and enthusiasm throughout this process. Professor Day gave us the opportunity to explore a topic we were passionate about in an animal model – zebra finches. Her guidance in working with zebra finches and her immense knowledge into neuroscience aided us throughout the thesis process. Finally, we are grateful for her master bird catching skills. Birds would likely still be flying around our lab had we not had her.

Beside our advisor, we would also like to express our deepest appreciation for all sixteen of our birds (even if they would sometimes get free and evade us). We also like to thank Professor Wally and the Whitman Psychology Department for supporting us in our neuroscience research. While we were unable to perform some steps in our experiment, we appreciate the department's willingness to fund a multidisciplinary project.

Abstract

Depression, the leading cause of disability worldwide, is often associated with social isolation. To explore this relationship, we placed zebra finches (*Taeniopygia guttata*) in isolation. Like humans, zebra finches are gregarious. We examined how prolonged isolation influences levels of corticosterone, a stress hormone elevated during depression, as well as time spent singing and body weight. Birds were randomly selected to live in either a social colony or isolated cage. We examined how fluoxetine, a selective serotonin reuptake inhibitor (SSRI), counters the effects of isolation. SSRIs raise serotonin levels in the brain, decreasing depressive symptoms. Inconsistent with previous literature, fluoxetine and environmental condition did not lead to significant changes in weight or birdsong frequency. With this said, we did see trends in our data supporting previous findings. For instance isolated birdsong frequency decreased and social birdsong frequency increased between pre-isolation and post-isolation. Additionally, after drug administration, birds given fluoxetine saw an increase in birdsong frequency, while birds given saline saw a decrease in such. In terms of weight, isolated birds overall experienced more weight fluctuation throughout the study than that of the social birds. Also as expected, birds administered fluoxetine showed fewer weight fluctuations than did birds on saline. Unfortunately, the coronavirus pandemic prevented us from collecting corticosterone concentration measurements. Our experiment, although filled with limitations, provides insights into the development of mental illnesses in isolated individuals and explores the benefits and costs of antidepressants.

Keywords: depression, social isolation, zebra finches, SSRI, weight, birdsong frequency, corticosterone

Introduction

Depression

Depression is a debilitating disorder suffered by people of various cultures, socioeconomic statuses, and ages. According to the World Health Organization (2020), over 264 million people across the globe suffer from depression, with lifetime prevalence rates ranging between 7-20% (Hischfeld & Weissman, 2002). Depression is not only hard to endure but also leads those affected to either think of or attempt suicide. In fact, suicide is the leading cause of death for 15-29-year-olds; this is one of many reason as to why depression is the leading cause of disability around the world (World Health Organization, 2020).

Overall, those suffering from depression exhibit common symptoms like changes in mood, such as sadness, and shifts in psychophysiological processes, such as disturbances in sleep. However, there are different types of depression (Belmaker & Agam, 2008). Depression is commonly split into two subcategories: endogenous and exogenous depression (Schimelpfening, 2019). Endogenous depression is experienced without an apparent external cause. In contrast, exogenous depression is triggered by a specific event, such as the loss of a loved one or job. Furthering the difference, Andus et al (2010) found that these two forms of depression involve abnormalities in different molecular mechanisms, making them think the two require different treatments. However, the specific treatments that work for one but not the other have not been determined yet, indicating an area where more research needs to be conducted. In the past, researchers speculated that because exogenous depression only reflected an external problem, it only needed treatment in the form of therapy (Hirshfeld, 1981). However, the altered molecular mechanism found by Andus et al (2010) suggests there is something biological/internal about exogenous depression.

Impact of Social Relationships on Depression

A multitude of factors contribute to exogenous depression, one being a personal feeling of an inadequate amount of social relationships (Holt-Lunstad, Smith, & Layton, 2010). Social relationships are a big part of human life. Researchers have found that people with stronger social relationships had a 50% greater likelihood of survival than those with weaker social relationships. In fact, lack of social relationships is correlated with mortality at similar levels as smoking and alcohol consumption, and is even more correlated with mortality than physical inactivity and obesity are. Researchers across disciplines have many explanations, including behavioral, psychosocial, and physiological reasons as to why social relations are so influential (Umberson & Montez, 2010). Social relationships provide a person with support, ultimately making a person feel less isolated and lonely, and therefore experience less of a depressed mood. For example, people that report they are unhappy with their social relationships also report higher, unhealthier levels of alcohol consumption (Cacioppo, Hawkley, & Berntson, 2003). Individuals that feel they lack enough social relationships feel they do not have strong support groups, ultimately making them more prone to use addictive substances, such as alcohol to deal with their problems. Therefore, social relationships are key to a person's well-being.

Social Isolation vs. Loneliness

While social isolation and loneliness are often used synonymously, they are not the same. Social isolation and loneliness are correlated ($r = 0.39$); however, social isolation refers to the complete absence of social relationships, whereas loneliness refers to a personal feeling of not having one's quality or quantity of relationships fulfilled (Mathews et al, 2016). Thus, a person who is not surrounded by others is not necessarily lonely. People have different affinities for social interaction, with some people, labeled as introverts, preferring lower levels of such interactions (Eysenck, 1947). Ultimately, both social isolation and loneliness are significantly correlated with depression, with loneliness ($r = 0.38$) being more so than social isolation ($r = 0.21$) (Mathews et al, 2016). When a person is depressed, they often withdraw themselves from others (Girard et al, 2013). Such a person becomes even more socially isolated, resulting in worsening depression.

Social Isolation and Depression

Social isolation and depression appear to share many symptoms. Social isolation is associated with higher hypothalamic pituitary adrenocortical (HPA) axis activation (Cacioppo, Capitanio, & Cacioppo, 2014). The HPA axis is essential in helping organisms cope with environmental challenges through the secretion of glucocorticoids, specifically corticosterone and cortisol (Herman et al, 2016). The HPA axis is primarily driven by neural mechanisms which invoke the release of corticotropin releasing hormone (CRH) from the hypothalamic paraventricular nucleus. CRH acts on the anterior pituitary resulting in the release of adrenocorticotropic hormone, which then acts on the adrenal cortex to produce corticosterone or cortisol, depending on the organism. Typically, the HPA axis, and its associated stress response, is beneficial to the survival of the organism; however, chronic stress and hyperactivity of the HPA axis can have negative effects on the body (Stephens & Wand, 2012). In fact, increased activity of the HPA axis and the resulting elevated concentrations of cortisol in the blood, are found in many depressive patients (Yang et al, 2015).

Social isolation is also associated with higher blood pressure and inflammatory control, lower immunity, and irregular sleep (Cacioppo, Capitanio, & Cacioppo, 2014). Further, animal studies of social isolation have shown decreased neurogenesis, brain-derived neurotrophic factor (BDNF), and nerve growth factor (NGF) in the hippocampus. The prefrontal cortex has lower glucocorticoid receptor (GR) and higher corticosterone levels in isolated animals, while the ventral striatum has lower cAMP response element binding protein (CREB). Finally, the amygdala has lower cell proliferation in isolated animals.

All of these symptoms of social isolation are also symptoms of depression (Mondal & Fatima, 2019; Rubio-Guerra et al, 2013; Kellerr et al, 2017; Leonard, 2010; Motivala, Sarfatti, Olmos, & Irwin, 2005; Anacker, Zunszain, Carvalho, & Pariante, 2011; Cowen, 2002; Blendy, 2006; Castro et al, 2010), demonstrating the high correlation between social isolation and depression.

Use of SSRIs in Depression

Another standard physiological indicator among socially isolated and depressed individuals involves the neurotransmitter 5-hydroxytryptamine (5-HT), better known as serotonin. Serotonin is popularly known for contributing to feelings of well-being and happiness and has been shown to be down-regulated in many psychiatric disorders, including depression, anxiety, and obsessive-compulsive disorder (Jonnakuty & Gragnoli, 2008). Interestingly, serotonin levels in socially isolated mice were also found to be lower than in non-isolated mice (Sargin, Oliver, & Lambe, 2016). Prolonged social isolation led to less active serotonin-producing nerve cells in the isolated mice than that in non-isolated mice. Overall, the decrease in serotonin in both isolated and depressed organisms suggests that serotonin can act as a biomarker for depression in isolated organisms.

In order to alleviate depression, serotonin has been targeted in antidepressants, specifically through selective serotonin reuptake inhibitors (SSRIs). Antidepressant usage in America has increased drastically, increasing by around 400% from 1988 to 2008 in people over the age of 12 (Pratt, Brody, & Gu, 2017), with 12.7% of such population taking them by 2014 (Winerman, 2017). SSRIs are the most common antidepressant prescribed, representing a big reason prescription of antidepressants has increased so much (McGlashan, Drummond, & Cain, 2018). SSRIs are so widely used because they are just as effective as other antidepressants, while also having less negative side effects. SSRI's work by blocking the reabsorption or reuptake of serotonin into neurons. This makes serotonin more available, active, and improves the transmission of messages between neurons.

Evidence suggests that SSRIs not only affect serotonin levels in the body but also have the potential to alter cortisol secretion. Cortisol is the body's main stress hormone which works with certain parts of the brain to control mood, motivation, and fear. The hormone is produced through the HPA axis, a pathway that is often hyperactive in major depression (Otte et al, 2016 as read in Ronaldson et al, 2018). Treatment with SSRIs increases serotonin, ultimately decreasing cortisol levels (Vythilingam et al, 2004), showing one-way serotonin acts to alleviate depressive symptoms (Porter et al, 2004 as read in Ronaldson et al, 2018).

Groups at Greater Risk for Social Isolation/Loneliness

One group especially at risk for social isolation and loneliness is prisoners confined in solitary confinement (Arrigo & Bullock, 2008). Prisoners in solitary confinement live in a small 6 x 8 foot cell for 22-23 hours per day, with minimal social interactions. Haney (2006) found that prisoners in long-term solitary confinement had a greater likelihood to experience mental illness symptomologies, including depression.

Another group at greater risk of social isolation and loneliness is the elderly. Pinquart and Sorensen (2001) found older age, specified as over 80 years of age, was directly related to feelings of loneliness. Interestingly, those 60-80 years old did not show greater levels of loneliness than the general population, like those over 80 did. There are different reasons why this is the case. For one, people over 80 lose loved ones at greater rates than younger people do due to ties with other older people, and thus have fewer social connections and are more likely to live alone (Singh & Misra, 2009). They are also more physically impaired, making travel to friends or family less frequent, as well as opportunities to keep old relationships and form new ones. Remembering the importance of social interactions, it is logical that loneliness has been associated with depression in the elderly. One study found that 83% of depressed seniors reported feeling lonely, compared to just 32% of non-depressed seniors (Holvast et al, 2015). Depression and loneliness' relationships can have a drastic impact on the general welfare of the elderly population. Depression has been shown to directly stimulate the body to produce inflammatory cytokines, chemicals widely implicated in several aging-related disorders such as cardiovascular disease, arthritis, type 2 diabetes, cancer, osteoporosis, periodontal disease, frailty, and functional decline (Kiecolt-Glaser & Glaser, 2002). Depression is also associated with a less active immune system. These conditions brought about by depression are especially dangerous for the elderly, increasing the elderly's morbidity and mortality. Ultimately, the authors concluded that while depression is multicausal, decreasing elderly isolation and feelings of loneliness would greatly reduce elderly depression and lower their morbidity and mortality.

Elderly and prisoners are not the only groups at greater risk for feelings of social isolation and loneliness. Other groups include those with more instances of victimization (Grossman, D'augelli, & O'connell, 2001), those with lower competencies, those who are female, those who are young (Pinquart & Sorensen, 2001), and those who participate in high amounts of social media (Primack et al, 2017). Social isolation and feelings of loneliness have increased over the last century (Hobbs & Stoops, 2002), seemingly becoming a trend in America. For example, in 1900, the average American household had seven people living in it, while the year 2000 had just two. Also, married households declined from representing 75% of American households in 1950 to just one-half of such in 2000. Overall, certain populations most at risk for social isolation and loneliness require additional care; however, as the examples have shown, it is unknown whether treating exogenous depression with drugs is beneficial. More analysis of the relationship between social isolation and exogenous depression is needed in order to approach such a problem.

Animal Psychological Illnesses

Humans are not the only species to experience depression and other forms of mental illnesses. For instance, dogs can develop canine cognitive dysfunction, the canine analog of human Alzheimer's Disease (Dewey, Davies, Xie & Wakshlag, 2019), as well as separation anxiety, and phobias (Sherman & Mills, 2008). Other, more exotic, pets have also shown signs of psychological problems. Cockatoos and African Gray parrots are known to express mutilating behavior when distressed. Mutilation often begins in an attempt to gain more attention, but if a laceration is deep enough to cause tissue or nerve damage, the bird will chew at its flesh even more (Davis, 1991). Additionally, feather picking in birds resembles human obsessive-compulsive disorder. While it is normal for birds to pull feathers from their breasts or thighs, birds with a feather picking problem tend to over exaggerate this grooming behavior. This behavior has been associated with loneliness, separation anxiety, and attention seeking behaviors (Mertens, 1997). Additionally, similar to humans, birds that exhibit this anxious behavior may also eat less resulting in weight loss (Pet MD, 2019).

Animal Stress Responses

Animals respond to chronic stress in a similar way. For instance, captivity of wild animals, a chronic stressor, has been observed to alter the HPA axis by changing the baseline HPA function and glucocorticoid secretion (Baker, Gemmell, & Gemmel, 1998 as read in Lattin, Bauer, Bruijn, & Romero, 2012). Like humans, chronic stress and continuous activation of the HPA axis in animals can result in immune and reproductive suppression as well as metabolism disruptions. Seasonal and chronic stress interact to affect HPA axis functioning with captive sparrows showing elevated baseline corticosterone levels and decreasing body weight after five days in captivity (Lattin, Bauer, Bruijn, & Romero, 2012). Drugs also used by humans have been used to improve animals' psychological health. For instance, fluoxetine, an SSRI, lowered feather picking behaviors in birds that had been doing such (Mertens, 1997). Additionally, Sperry et al (2003) examined aggression in zebra finches and found that fluoxetine injections led to lower corticosterone levels.

Why Zebra Finches?

Our study chose to use zebra finches (*Taeniopygia guttata*) as an animal model because, like humans, they are gregarious and appear to be affected by social relationships, or lack thereof. Furthermore, zebra finches have similar stress pathways to humans. Both organisms have and use the HPA axis pathway. As described above, the HPA axis is activated in organisms that are isolated and/or depressed.

Like humans, social interaction appears to play an important role in animal health and behavior. Specifically, zebra finches are known for being gregarious. Male and female zebra finches live colonially and are socially monogamous. Many bird species are socially monogamous in that they form lasting relationships with a partner; however, monogamy can take on a variety of different forms. Some species are serially monogamous (they take new partners every season) while others, including zebra finches, are pair-bonding (mate for life) (Remage-Healey & Adkins-Regan, 2003 & Adkins-Regan & Tomaszycki, 2007). Previous studies have evaluated how pair bonds affect behavior. For example, a female and male zebra finch were paired for two weeks and then separated. Researchers then expose the female to the same or novel male to see how pair bonds affected future interactions with other birds. Females displayed higher levels of behaviors associated with pair-bonds when presented to their mate rather than a novel male (Svec, Licht, & Wade, 2009). Also, separation of paired zebra finches results in elevated circulating corticosterone, a hormone that increases in response to stress (Banerjee & Adkins-Regan, 2011). A study examining zebra finch's behavioral and neuroendocrine responses to pair mate separation found that plasma corticosterone concentrations were elevated during pair mate separation and reduced upon reunion with their mate (Remage-Healey & Adkins-Regan, 2003). This suggests that lack of social interaction, particularly with mates, can affect birds' health.

Birdsong

Communication and vocal signals are crucial for some organisms' survival and reproduction success. Specifically, zebra finches rely heavily on birdsong in finding a mate. Like human children, young zebra finches learn to vocalize by imitating an older individual, typically the father. Zebra finches are known to exhibit a "sensitive period" for development of song similar to humans "critical period" for developing language. Interference during this period results in permanent impairment (Gobes, Jennings, & Maeda, 2019). Overall, a main role for male birdsong is attracting females and forming monogamous pairs. Female birds use male birdsongs as an honest signal of sexual fitness (Day, 2019). Dopamine appears to play a role in the formation of monogamous pairs. Research shows that birdsong induces dopamine in the striatum of mated or single male zebra finches, while only mated females exhibit reinforcement when they hear the song exclusive to their mate. Such sex specific responses might explain why such gregarious animals are able to maintain monogamous pairs (Tokarev et al, 2017).

Weight

It is normal for body weight to fluctuate throughout the day (Lissner et al, 1991). Some reasons to account for such a phenomenon, including diet (food and fluid intake), exercise schedule, time of day, and the consumption of SSRIs (Gibson-Smith et al, 2000). The authors Wannamethee, Shaper, and Walker (2002) determined up to four percent weight fluctuation signifies a stable weight fluctuation, indicating healthy physiology. However, weight fluctuations above four percent represent a physiological problem. One such cause of greater weight fluctuation is if someone is suffering from depression/stress (Foreyt et al, 1995). Weight gain (Maina, Albert, Salvi, & Bogetto, 2004) and loss (Blundell & Halford, 1998) are both symptoms of depression/stress. With many people taking SSRIs to alleviate depression, weight fluctuation could take two forms. One could entail a person's weight fluctuating more because of the SSRIs being consumed. The other possibility could be a person's weight fluctuating less because of the alleviation of depression. Gibson-Smith et al (2000) found the latter to be true, determining depression to be the main factor in weight fluctuation over the effect of SSRIs.

Purpose of Study

The effects of social isolation on stress processing appears to have implications on those prone to depression. The purpose of our study was to explore the relationship between social isolation and depression in an animal model to determine whether SSRIs can be used to mediate these effects. We randomly selected male zebra finches to either live in an isolated or group cage to examine how prolonged isolation influences corticosterone levels, time spent singing, and weight fluctuations. These variables will provide a measure for depression, with corticosterone showing stress levels, birdsong frequency showing diminished interest, and weight gain or loss (a sign of depression in humans) showing lack of motivation. Midway through the experiment, fluoxetine was administered to one-half of the animals, in both the isolation and colony groups, to test whether an SSRI could counter the negative effects of isolation. Based on past research which found that when placed in isolation, zebra finches expressed greater levels of corticosterone (Perez et al, 2012) we hypothesized that birds in isolated cages would have higher corticosterone levels (indicative of physiological stress), a more fluctuating weight, and reduced time spent singing than those of birds living in a social setting. Additionally, based on what we know about antidepressants and the pathways they affect, we hypothesized that fluoxetine would reduce effects of isolation resulting in lower corticosterone levels, an increase in birdsong frequency, and a more stable weight, based on the findings of Gibson-Smith et al (2000). These experiments have implications not only on how singing behavior in zebra finches, an essential model for human speech, may differ in different caged environments, but also how social isolation could play a role in the development of mental illness. Furthermore, our study explores the possibility of using SSRIs to reduce stress during social isolation.

Methods

Subjects

We used zebra finches bred in the psychology lab at the University of California, Los Angeles. Subjects were 16 male zebra finches. The mean age of the subjects was more than 150 days post hatch. All the birds had free access to water and food (mixed seed, grit to aid digestion, cuttlebone, and calcium powder supplements). Additionally, once a week, all birds received a vegetable, hard-boiled egg, millet spray, and water bath. Cages were cleaned weekly by removing debris and bird excrement, and relining the bottom of the cage with newspaper. The temperature in and around the cages was maintained between 50°F to 85°F, and if the humidity dropped below 10%, we used water baths or a mister to increase it. The light condition used was 14:10 h light:dark, turning on at 8:00 A.M.

Procedures and measurements

Social Isolation

For at least one month before the experiment, all subjects (n = 16) were housed in a group cage. At the conclusion of this pre-isolation period, eight out of 16 birds remained in the group cage, and the others were housed in individual chambers situated within sound attenuation chambers that ensured visual and auditory isolation.

Drug Administration

10 mg of fluoxetine was purchased from Sigma Aldrich (F132), dissolved in 10 ml of saline, and equally aliquoted into 10 microcentrifuge tubes. In order to dose the

birds with the desired 2 mg/kg, birds were given 2 μL for every gram the bird weighed. Drug administration began with weighing the birds, and then pipetting the respective amounts into the beaks of each bird. Following six weeks of isolation or group housing, four birds in the isolated chambers and four birds in the group cage were dosed everyday for two weeks. The rest of the birds in the isolated and group cages were dosed with saline (0.9% NaCl, 2 μL/gram of bird) as a placebo everyday for two weeks (Merck Vet Manual, 2019).

Song recording

To measure the birdsong frequency of the zebra finches, we used microphones connected to the computer program Sound Analysis Program. The subjects in the group cage were temporarily removed from the group and housed individually overnight to record songs until the following afternoon. The subjects in the isolated chambers had their microphones attached inside of their cage/chamber. At the conclusion of the experiment, we analyzed the data using MatLab.

Weight

The subjects were taken out of the cages and placed on a balance to measure their weight for 14 times points throughout the experiment: pre-isolation, post-isolation, 11 post-daily drug administrations, and post-drug.

Fecal sample collection

Fecal samples were always collected between 10:00 A.M. and 1:00 P.M. After two weeks in the colony setting, we collected fecal samples for a pre-isolation measure. Zebra finches were removed from their cage and placed into a separate smaller cage lined with aluminum foil. Birds stayed in such cages for 30 minutes or until there was visible fecal matter. Birds were transported back into their respective cages and the aluminum foil was rolled up and placed in 50ml falcon tubes prelabeled with the bird ID, time, date, and experimental condition of the bird. Fecal samples were then placed into the -80 °C freezer until we were ready for the hormone assay.

Hormone Assay

Hormone assays were performed using the ELISA method (Kit Corticosterone ELISA). Fecal samples were scratched off the aluminum foil into their respective tubes and then frozen using a lyophilizer for 14 to 18 hours to control for fiber and water content. The frozen fecal samples were ground up. We extracted 0.300 grams of ground feces and mixed it with 5 ml of 80% ethanol and finally vortexed it for 30 minutes at 15000 rpm. After we centrifuged and aliquoted out each supernatant, we diluted the sample 1:10 with an assay buffer for 15 minutes at 2500g. The fecal samples were frozen at -80°C until the ELISA was performed (Sheriff et al, 2010).

Statistical analysis

All statistical tests were performed using SPSS and Prism. We applied a 3 x 2 x 2 repeated measure design in our study. In this case the within group independent variable, time, had three levels (pre-isolation, post-isolation and post-drug). The housing (isolation vs. group) was between groups independent variable, while the injection (fluoxetine vs. saline) was within group variables. The dependent variables were corticosterone level, song frequency, and weight. Our design is appropriate to test our hypotheses that isolation and serotonin would interact, showing their effect on corticosterone level, song frequency, and weight. We also hypothesized a main effect between the dependent variables corticosterone, song frequency, and weight, and the independent variables social environment and drug condition.

Results

Sixteen male zebra finches were bred at University of California, Los Angeles and sent to Whitman College in Walla Walla, WA. All birds were considered mature adults (>150 days post hatch). Experiments were performed under the authorization of Whitman's Institutional Animal Care and Use Committee according to the guidelines of the Federal Office of Laboratory Animal Welfare.

To investigate how social isolation affects weight and birdsong frequency, birds were randomly selected to be in one of two environmental conditions: social/frat or isolated. Birds in the social condition were housed with seven other males in a room with approximately 50 finches; birds in isolation were housed individually within sound attenuation chambers that prevented visual and auditory interactions with other birds. Once in their environmental condition, they were randomly assigned to the saline or fluoxetine drug condition.

Three main time points during the experiment were used: pre-isolation, post-isolation, and post-drug. Each of these periods lasted two weeks. During the pre-isolation period, we housed all of the birds together in a large cage labeled "Frat." These two weeks were meant to give birds time to get accustomed to their new environment. Then we selected 8 birds randomly from the frat and moved them into isolated cages. Overall, we collected our data (weight and song) at 14 different time points over 6 weeks.

Weight

Changes in weight are observed in depressed individuals so antidepressants like SSRIs can work to stabilize weight through alleviating depression (Gibson-Smith et al, 2016). To determine whether prolonged social isolation with or without SSRI treatment led to weight fluctuations, we weighed birds at the various experimental time periods. We ran a repeated measures ANOVA and found that there was a main effect of time on weight, $F(11, 132) = 3.178$, $p = 0.001$, partial $\eta^2 = .209$. However, there was no main effect of environmental condition on weight, $F(11, 132) = 1.356$, $p = 0.201$, partial $\eta^2 = .102$, or of drug condition on weight, $F(11, 132) = .643$, $p = .669$, partial $\eta^2 = .051$.

The average weight of the birds over the course of the experiment (pre-isolation to post-drug) was 13.0 ± 0.946 g. (mean ± SD). The average weight fluctuation across this time was 0.265 ± 0.267 g., translating to an average percent fluctuation of 2.03% g per day weight was recorded.

There was a main effect of time on weight. In addition to observing a main effect, we also saw some interesting trends in our data. The average pre-isolation weight of the birds in the social condition (13.5 ± 1.2 g; mean ± SD) was higher than that of birds in the isolated condition (12.6 ± 0.83 g) (Figure 1). This trend continues over the following time points; during the post-isolation and post-drug time points, respectively, the average weight of the social birds (13.1 ± 1.1 g, 13.4 ± 1.2) remained greater than those of the isolated birds (12.5 ± 0.66 g, 12.5 ± 0.79).

Furthermore, there was no main effect of environmental conditions on weight. In order to look at the weight fluctuations across the experiment, we plotted weight vs time for our different conditions. We looked at R^2 values in order to see how weight measurements varied across the timepoints. Smaller R^2 values indicated greater weight fluctuations. We calculated the R^2 for every individual bird across various time points, and then took average R^2 values for the four environmental (social vs isolated) and drug (saline vs SSRI) conditions. The isolated birds (R^2=0.165) had greater weight fluctuations from the start of the study to the end (pre-isolation to post-drug) than that of the social birds (R^2=0.275).

In regards to the drug condition, there was not a main effect of drug condition on weight. With this said, the average weight of the 5-HT birds (13.2 ± 0.89 g, 13.2 ± 0.84, 13.2 ± 1.1) remained greater than that of the saline birds (12.9 ± 1.3 g, 12.5 ± 0.89, 12.4 ± 1.2) across the pre-isolation, post-isolation, and post-drug time points respectively. The birds administered saline (R^2=0.270) had greater weight fluctuations compared to that of the birds administered 5-HT (R^2=0.415) over the 12 dosage dates (post-isolation to post-drug) (Figure 2).

Finally, there was no interaction between environmental condition and drug condition, $F(1, 12) = 1.495$, $p = 0.245$, partial $\eta^2 = 0.111$ (Figure 3 and 4). However, isolated birds administered 5-HT (R^2=0.536) had the least weight fluctuations compared to those of social/5-HT birds (R^2=0.294), social/saline birds (R^2=0.278), and isolated/saline birds (R^2=0.261).

Birdsong Frequency Results

Overall, there was no interaction between environmental condition and drug condition, $F (2, 24) = 0.068$, $p = 0.931$, partial $\eta^2 = 0.006$. Additionally there no main effect of song frequencies across the time points, $F (2, 24) = 0.081$, $p = 0.919$, partial $\eta^2 = 0.007$, or a main effect of environmental condition on birdsong frequency, F (2, 24) =

1.261, p = 0.301, partial $\eta^2 = 0.095$, or of drug condition on birdsong frequency, F (2, 24) = 2.643, p = 0.093, partial $\eta^2 = 0.18$.

Throughout the experiment, we recorded vocalizations (songs and calls) from each bird at various time points. We calculated how much each bird sang (seconds of song sung between 8:00 A.M. and noon) at three main time points: pre-isolation, post-isolation and post-drug. Overall, the average song frequency of birds in the isolated condition began and remained higher than those in the frat (Figure 5). Pre-isolation, post-isolation, and post-drug average song frequencies for the isolated birds were 331.2 ± 292.1 s, 228.1 ± 252.6, 238.5 ± 206.9 respectively. The same for the social birds were 102.4 ± 200.8 s, 192.9 ± 153.1, and 145.4 ± 152.1 respectively. There was no main effect of song frequencies across the time points, F (2, 24) = 0.081, p = 0.919, partial $\eta^2 =$ 0.007.

Birds administered 5-HT had a greater average pre-isolation birdsong frequency (312.5 ± 314.8 s) compared to that of birds administered saline (121.1 ± 188.4 s). There were differences in trends between the two drug conditions going from pre-isolation to post-isolation, and then post-isolation to post-drug. Saline birds had an increase in their average birdsong frequency from pre-isolation to post-isolation, and then decreased from post-isolation (258.3 ± 237.3 s) to post-drug (173.3 ± 153.7 s). In contrast, average birdsong frequency decreased from pre-isolation to post-isolation in birds that received 5-HT, and then increased from post-isolation (162.8 ± 162.7 s) to post-drug (210.6 ± 215.5 s). There was no main effect on drug condition on birdsong frequency, F (1.958, 23.495) = 2.643, p = 0.093, partial $\eta^2 = 0.18$ (Figure 6).

Lastly, there was no interaction between environmental condition and drug condition, $F(1.958, 23.495) = 0.068$, $p = 0.931$, partial $\eta^2 = 0.006$ (Figure 7).

Corticosterone Results Prediction

Due to the coronavirus pandemic, we were unable to perform the ELISA corticosterone assay like expected. We predicted that at the pre-isolation time point, corticosterone concentrations would be similar for birds in each condition (Figure 10). After the isolation period, we hypothesized that birds in the social condition would have adjusted to their social cage environment and thus have lower corticosterone concentrations, indicative of lower physiological stress, while birds in isolation would be stressed and show higher corticosterone concentrations (Figure 8 and 10). Finally, at the post-drug time point, we expected birds dosed with fluoxetine to have lower corticosterone than their previous time point (Figure 9 and 10).

Discussion

The goal of the current study was to investigate the role of social isolation in mitigating depressive symptoms in zebra finches before and after the administration of selective-serotonin reuptake inhibitor (SSRI) antidepressants. We hypothesized that birds placed in social isolation would display more depression-like symptoms: a greater fluctuation in weight, lower birdsong frequency, and greater corticosterone concentrations compared to those of birds in the frat/group setting. We further predicted that birds administered serotonin (5-HT) would reverse these depressive symptoms, leading to a more stable weight, greater birdsong frequency, and lower corticosterone concentrations.

Overall, the present findings did not support our hypotheses. There were no significant main effects of social isolation or drug condition on birdsong frequency or weight (with the exception of time on weight). Further, the interaction between the environmental and drug conditions showed no significant changes in birdsong frequency or weight. The birds within each condition appeared to vary drastically to each other in birdsong frequency and weight over each time point. The variation likely would have appeared less drastic had we had more birds per condition.

Birdsong frequency over the three time points was not significantly different between social or drug settings. This was not in alignment with our hypothesis, which predicted that birds placed in isolation would display more depression-like symptoms and birds administered serotonin would see a reverse in depressive symptoms. Unexpectedly, birdsong frequencies varied drastically from the start of the experiment with birds in the isolated environment singing much more than birds in the social environment. This was surprising, as birds were randomly placed in isolation or in social settings. A possible reason for this disparity could be because of our “random selection” protocol in which the first eight birds we caught were assigned to the social condition. The birds' anxiety likely affected this “random selection” process. Capturing birds proved tricky and we were best able to catch birds that stayed in place the most. Animals commonly “freeze” when they are in an anxious state (Roelofs, Hagenaars, & Stins, 2010), so it is likely that we captured the more stressed birds first (social birds). This explains why birds in isolation sang more than those in the social environment prior to isolation. A more random selection process could have entailed us writing down all 16 bird IDs and then using a random number generator to assign conditions (i.e. 1 is social, 2 isolated).

Another reason for this large disparity in birdsong frequency at the pre-isolation time point could have been because the social birds had their birdsong frequency measured first. At the pre-isolation time point, all birds might have still been getting accustomed to their new environment in the lab, and consequently more stressed than normal. The social birds, designated to have their birdsong recorded first, had less time to get acclimated to the environment before they had their birdsong frequency measured. On the other hand, the isolated bird group had more time to adjust to their new environment before being transferred to the birdsong box. This extra time to adjust could have resulted in lower stress levels and, ultimately, more time spent singing. This problem arose because we did not have sufficient equipment to measure all 16 birds birdsong frequency at once. Had we been able to do this, we could have had more control over the social and isolated birds’ stress levels. This would have likely led to more equal birdsong frequencies at the pre-isolation time point.

Furthermore, social birds might have had a much lower average birdsong frequency at pre-isolation because of experimental error. Since the social birds were the first group that we recorded birdsong for, we likely made the most mistakes at this time. We all were new with handling birds and recording birdsong, so it took some time for us to get accustomed to the procedure. During the first round of recordings, birds often escaped their cages throughout the day. We would find birds outside of their recording cages where their birdsong could not be recorded accurately. While we learned from our mistakes by fixing cages and getting better at recording when it came time to collect isolated bird's pre-isolation data, these experimental errors likely had a grave effect on the pre-isolation, social birdsong data.

Interestingly, there was also a large variation in pre-isolation birdsong frequency depending on drug treatment. Saline birds had a much lower pre-isolation birdsong frequency than birds selected to receive 5-HT. This was a prominent abnormality as we had yet to select which birds were to be administered drugs at this point in the experiment. There should have been no difference between the birds in the different drug conditions at the pre-isolation time point. A larger subject pool would have reduced this variability.

Even though we did not achieve significant results, average birdsong frequencies for the environmental conditions did follow our predicted trends in pre-isolation and post-isolation. Across these time points, average birdsong frequency in the social environment increased, while the average birdsong frequency in the isolated environment decreased. Birds in the social environment may have become more accustomed to their new environment between these time points and thus sang more, indicating their stress levels had diminished. Alternatively, we had more practice and got better at handling them through the course of the experiment, which could have caused the birds to be less stressed. However, the isolated birds, by singing less, shows us that birdsong in the isolation condition was at least somewhat indicative of elevated stress behaviors.

Similarly, average birdsong frequencies for the drug condition followed our predicted trends. While not statistically significant between the post-isolation and post-drug time point, birds administered saline on average experienced a decrease in birdsong frequency, while birds administered 5-HT experienced an increase in birdsong frequency. Therefore, although not significant, dosages of 5-HT led to a decrease in stress as we predicted.

Our error bars (standard deviations) on the various birdsong frequency figures reflect great variation in birdsong frequency. While some of this variability is likely due to possible data collection errors, we were also new to analyzing birdsong frequencies and had few subjects. A lot of the birdsong analysis involved deciding what was and was not birdsong, something we had little to no practice in doing. More experience with such analyses would likely lead to our error bars decreasing, maybe leading to more significant results. Had one of us done all of the birdsong analysis, there might have been less variation, as we felt deciding what was and what was not birdsong was subjective. This would have taken a lot more time however. Additionally having only four subjects per condition likely contributed to our large error bars. With only four subjects, it was easy for one bird to skew the mean and contribute to large error bars.

In addition to birdsong frequency, our weight dependent variable was not significantly affected by social setting or drug condition. Analyzing weight, we found that birds chosen for the frat condition were heavier than the birds chosen for the isolation group at the start of the experiment. This was not anticipated as the birds were randomly selected into each condition. We think the initial disparity in weight may have transpired in a similar way the initial birdsong disparity occurred. As explained previously, we expect that birds caught first were more stressed, and therefore were more likely to freeze and be captured. Perhaps bird weight also played a role in our "random selection" as we likely had an easier time catching birds that were more stressed and larger.

Our weight hypothesis centered around the fluctuations of weight; the data again followed our predicted trends. Isolated birds had on average greater weight fluctuations than social birds. This can be explained by the isolated birds dealing with greater levels of stress. Knowing that a common symptom of stress/depression is weight fluctuation (Foreyt et al, 1995), we expected isolated birds to experience greater stress, and thus greater weight fluctuations. Furthermore, birds in the saline condition saw greater weight fluctuations than birds in the 5-HT condition. Saline birds, without the relief from the stress/depression, saw the greatest weight fluctuations, as they represented the most stressed/depressed birds.

Our results are not in alignment with previous findings that antidepressants like 5-HT cause side effects, specifically weight fluctuation. Serotonin has a prominent role in weight stability, both in causing weight loss (Blundell & Halford, 1998) and gain (Maina et al, 2004). Blundell and Halford (1998) found serotonin can prevent excessive consumption of food through its relation with leptin. Leptin, a hormone, binds to various cellular receptors, including two 5-HT receptor subtypes. Once bound, leptin functions by inhibiting hunger and thus causing weight loss. However, Maina et al (2004) reported the conflicting finding that serotonin leads to weight gain (Maina et al, 2004). Gibson-Smith et al (2000) also found serotonin causes weight gain, but also found weight no longer increased significantly when depression was taken into effect. Thus depression seems to be the most important factor to examine when measuring weight fluctuations. The birds on 5-HT saw less weight fluctuation than the saline birds did. This supports the previous studies as the 5-HT birds, since they are taking SSRIs, are less stressed/depressed than the saline birds. Therefore, diminishing depression being the main factor in weight fluctuation is shown to be true in our experiment also.

It is important to note that the average percent fluctuation per day weight was recorded of the birds of 2.03% would have fallen into the stable weight category of the study by Wannamethee et al (2002), as it is below the four percent cutoff point. SSRIs take time to have their effects (Machado-Vieira et al, 2010), so having the birds on 5-HT for more time would have likely led to greater weight differences among the different drug conditions. The specific timetable is not specifically known, although it is for sure longer than the two-week period we dosed the birds for. Machado-Vieira et al (2010) estimated the timetable to be closer to several weeks to months. However, the small weight changes that did occur could indicate there was a physiological stress response starting to pervade in the birds.

To fully examine the physiological response the birds experienced in social isolation with serotonin, we would have ideally finished out our study by performing the ELISA corticosterone assay. Unfortunately, the coronavirus pandemic prevented us from running this last step of our experiment, which would have assessed how corticosterone may have fluctuated during isolation and whether SSRIs had any effect on reducing corticosterone levels in birds. We predicted that all birds' corticosterone levels would be high at the beginning stage of the experiment because birds would be stressed because they were suddenly separated from the females they were brought in with. Then their corticosterone concentration would decrease after they acclimated themselves to their cages. Birds in the isolated group would have increased corticosterone levels and birds in the frat group would have more stable or even decreased corticosterone levels. At the post-drug point, the isolated birds dosed with fluoxetine would have a greater decrease in corticosterone levels than that of the frat birds dosed with fluoxetine. The isolated birds dosed with saline would have small increases in corticosterone concentration, and the frat birds dosed with saline would have small decreases in corticosterone concentration. While there is the possibility that the finches experienced insignificant changes in corticosterone during this study, future research should examine our methods and continue to explore the effects of social isolation on depression.

Future experiments should take into account the assumption that birds and humans respond to isolation in similar ways. Although studies have shown that birds can experience feelings, have empathy, and grieve for the death of a partner, it is unclear whether birds are an accurate model of human emotion and behavior (Vignieri, 2015). With more funding and resources, animal models may be better fits for these kinds of study. Additionally, more subjects would add to the power of the study, therefore reducing the probability of type II errors and producing more noticable findings. Overall, had we been able to choose an animal model with emotional responses comparable to that of humans or increase the number of subjects in our experimental pool, we might have been able to see a more significant change in weight and song.

In addition to our subject pool, we were also limited with time. If we had more time, we would have allotted longer periods between each measurement. One reason why more time would have been beneficial was because measuring birdsong, in itself, often took up to five days as we waited for the bird to sing at least once before beginning recording. Five days in a song recording box out of the average 15 days in between each measurement could have profound effects on our data.

This said, further research into the effects of social isolation on depression are of great importance. Social isolation and depression share many physiological characteristics including the stimulation of the hypothalamic pituitary adrenal (HPA) axis. While the natural stimulation of the HPA axis in response to stress is beneficial, chronic stress and hyperactivity of the axis can have negative effects on the body. With this correlation in mind, it is thought that inadequate social relationships contribute to exogenous/situational depression. Past research has revealed the importance of social relationships on human life - specifically, those with strong social relationships have a 50% greater likelihood of survival than those with weaker relationships. Positive social relationships provide people with support, ultimately lessening feelings of isolation and decreasing depressed moods (Cacioppo, Hawkley, & Berntson, 2003).

Beginning this study, elderly and prisoners in solitary confinement were two social groups we viewed as being of greater risk for social isolation and consequential depression. Now, however, we can expand this at risk group to almost everyone. The coronavirus outbreak has forced many to adjust their lives to align with social distancing standards and stay at home orders. For many people social distancing, defined as "keeping space between yourself and other people outside of your home" (CDC), is anything but normal. No longer going to work, meeting with friends at restaurants, or attending gatherings causes a significant loss in social connections. While most people have ways to stay in touch with people, be it through call, video chat, or social media, social distancing can feel isolating. At this time, people are not only physically separated from one another, but also increasing their use of social media which has been associated with feelings of social isolation (Primack et al, 2018). It will be interesting to see how the increase in social distancing, and corresponding increase in social isolation, affects rates of depression in the United States and world.

Our findings also offer insights into the differences between endogenous and exogenous depression. Although we were not able to conduct the ELISA and confirm the isolated birds experienced full on depression/elevated stress response, the elevated stressful behaviors indicates the birds did experience at least partial depression/stress. Similarly, the birds that received 5-HT saw indications of a reduction in their stress, indicating antidepressants played a role in decreasing such stress responses. This said, our results saw enormous variation and insignificant p-values, making it difficult to conclude anything. We cannot say confidently that our results show social isolation leads to depression or that antidepressants can be used to relieve isolation-based depression.

It is possible that isolation-based depression, a form of exogenous depression, is not treatable solely by antidepressants like an endogenous form of depression. Before the abrupt end of our study, we had thought about adding a fourth time point - reacclimation. The reacclimation process would have involved selecting four of the eight isolated birds (two on 5-HT, two on saline) and reacclimating them into the social setting. Then, we would have compared the reacclimated birds with the birds still in isolation, looking at their birdsong frequencies, weights, and corticosterone levels. It would have been interesting to compare these levels to see if reacclimation offered a better treatment outcome then 5-HT did. Had we carried out this experiment and gotten such results, we would have been able to conclude exogenous and endogenous depression need to be treated as different disorders. These findings would have contributed to the results of Malki et al (2014), who observed different gene expressions between the two types of depression. Additionally it would support the results of Andus et al's (2010) who found that endogenous and exogenous depression have different molecular mechanisms. We could then conclude that endogenous and exogenous depression are different and require very different treatment options.

With this in mind, more research needs to go into the physiology and treatment of exogenous depression. The majority of studies on depression focus on Major Depressive Disorder (MDD), a condition characterized by lower levels of serotonin (Blier & Mansari, 2013). MDD is often associated with endogenous depression and thus commonly treated with 5-HT; however, there might be better treatment options, other than 5-HT, for exogenous depression. The isolated birds dosed with fluoxetine might have experienced a reversal in depressive symptoms had they been treated with some other type of drug or been placed within a different situation. Treating the situation for exogenous depression would make sense as exogenous depression is commonly referred to as situational depression. Although it is not always possible to fix depressing situations such as after the death of a loved one, prescribing antidepressants might not always be the best treatment option. Antidepressants have many negative side-effects, with 38% of people reporting them (Cascade, Kalali, & Kennedy, 2009). The most common reported in this study were problems with sexual functioning, weight gain, and sleepiness. The development of new treatment options, such as other drugs or more cognitive-behavioral treatments like therapy, might be more advisable for people in such situations.

Figures and Tables

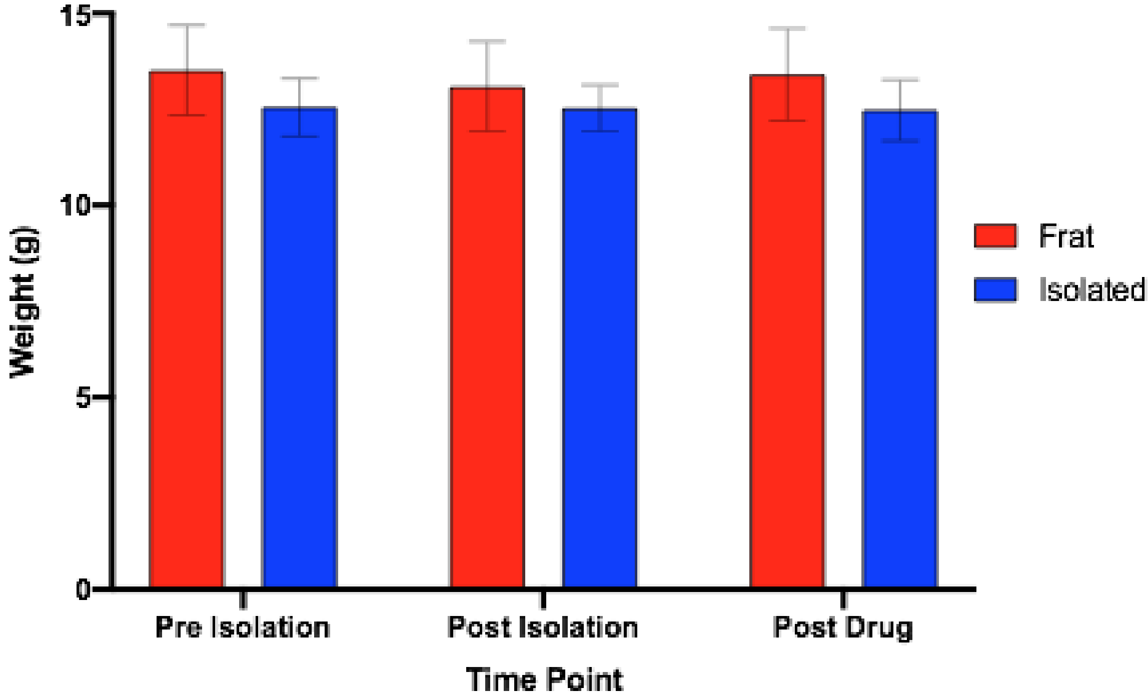

Figure 1. Average weight of birds in each environmental condition over three time points. Error bars refer to the standard deviations of each condition at each respective time point.

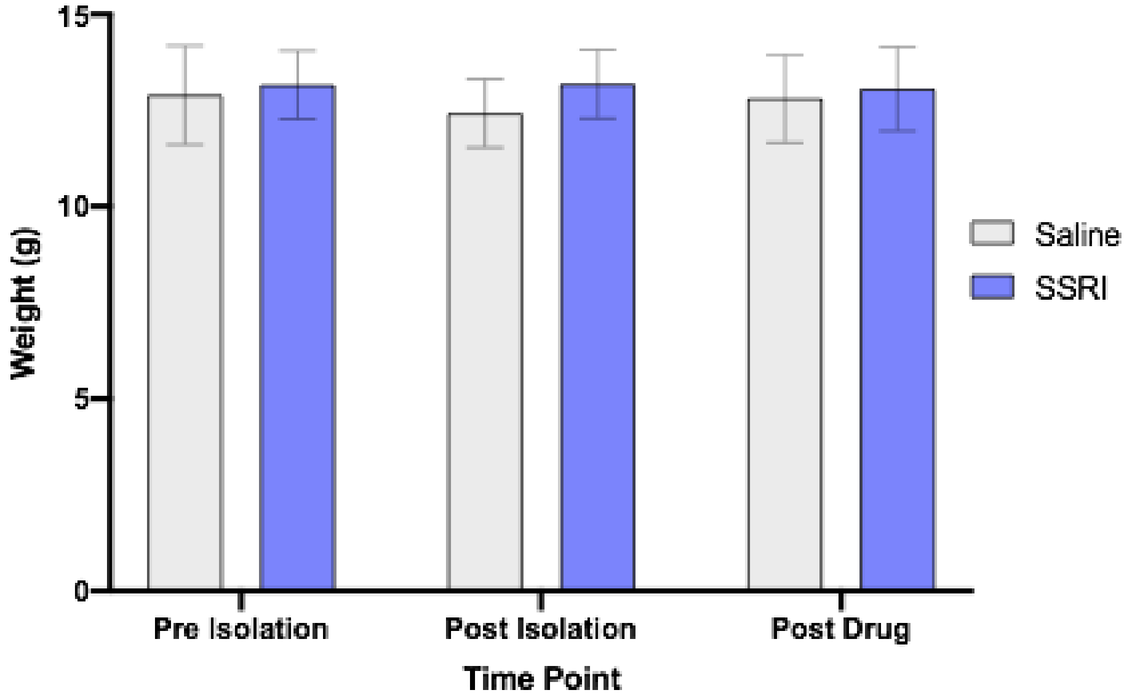

Figure 2. Average weight of birds in each drug condition over three time points. Error bars refer to the standard deviations of each condition at each respective time point.

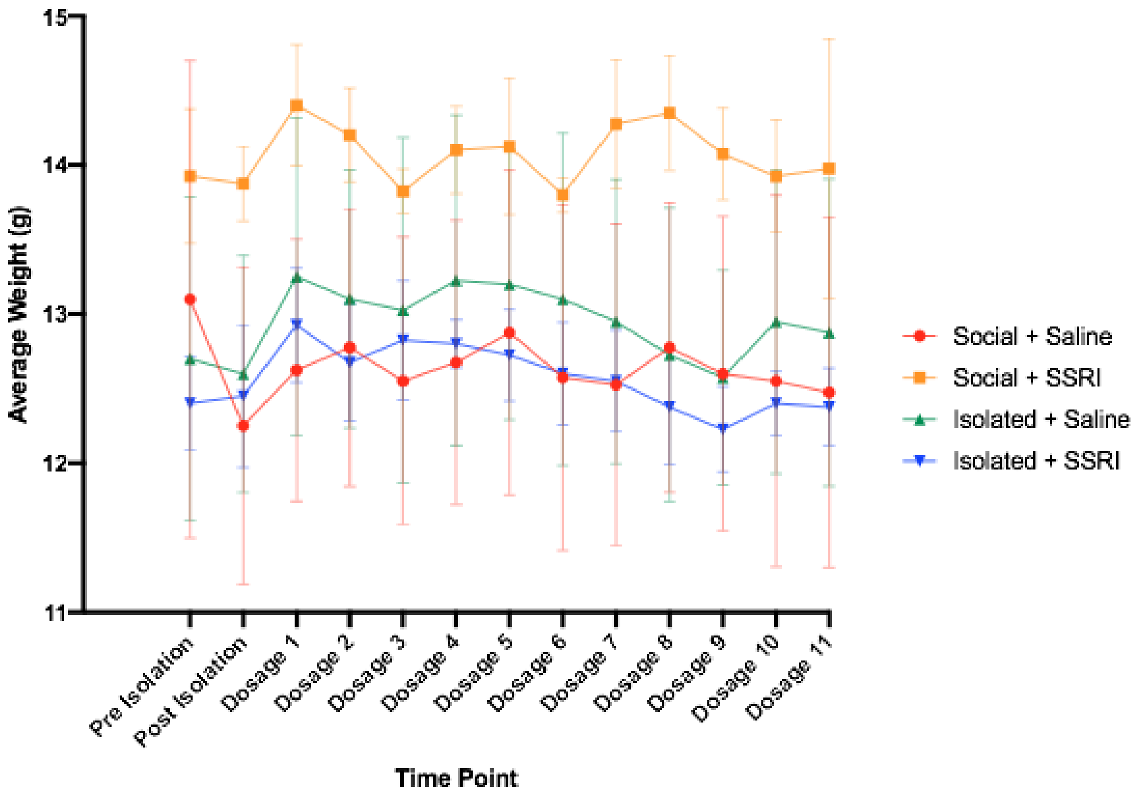

Figure 3. Average bird weight among different conditions across the various time points. Error bars refer to the standard deviations of each condition at each respective time point.

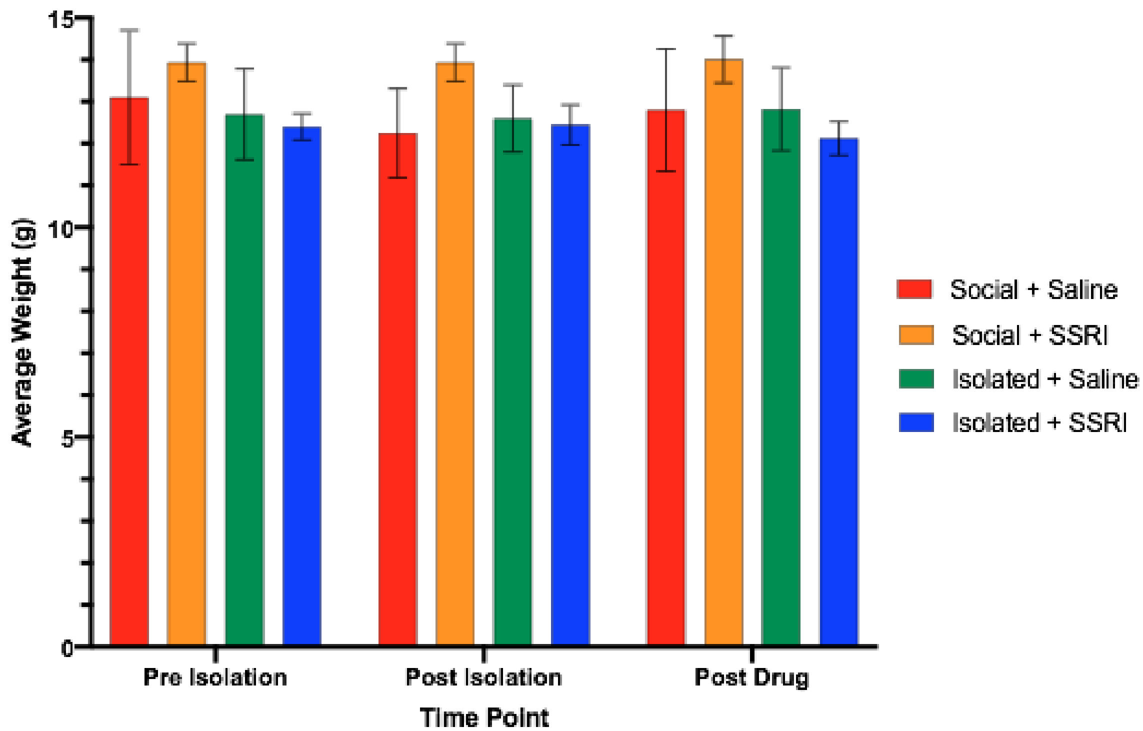

Figure 4. Average weight of birds in environmental and drug condition over the three time points. Error bars refer to standard deviations of each condition at the respective time point.

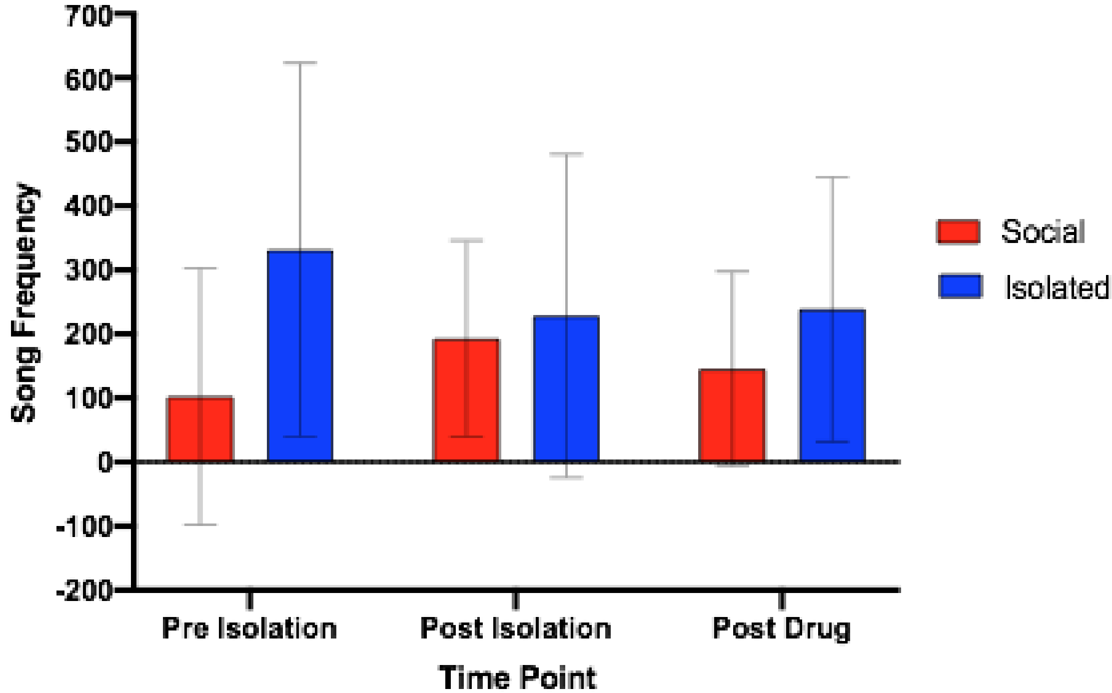

Figure 5. Average song frequency sung by birds in each environmental condition over three time points. Error bars refer to standard deviations of each condition at the time point.

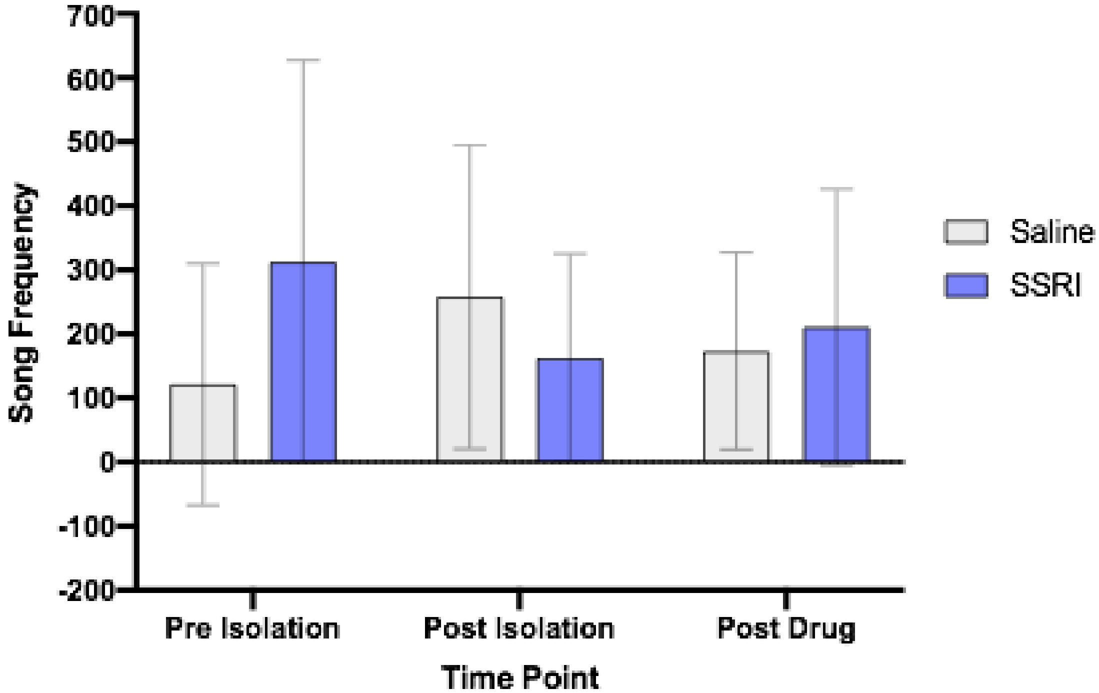

Figure 6. Average song frequency sung by birds in each drug condition over three time points. Error bars refer to standard deviations of each condition at the time point.

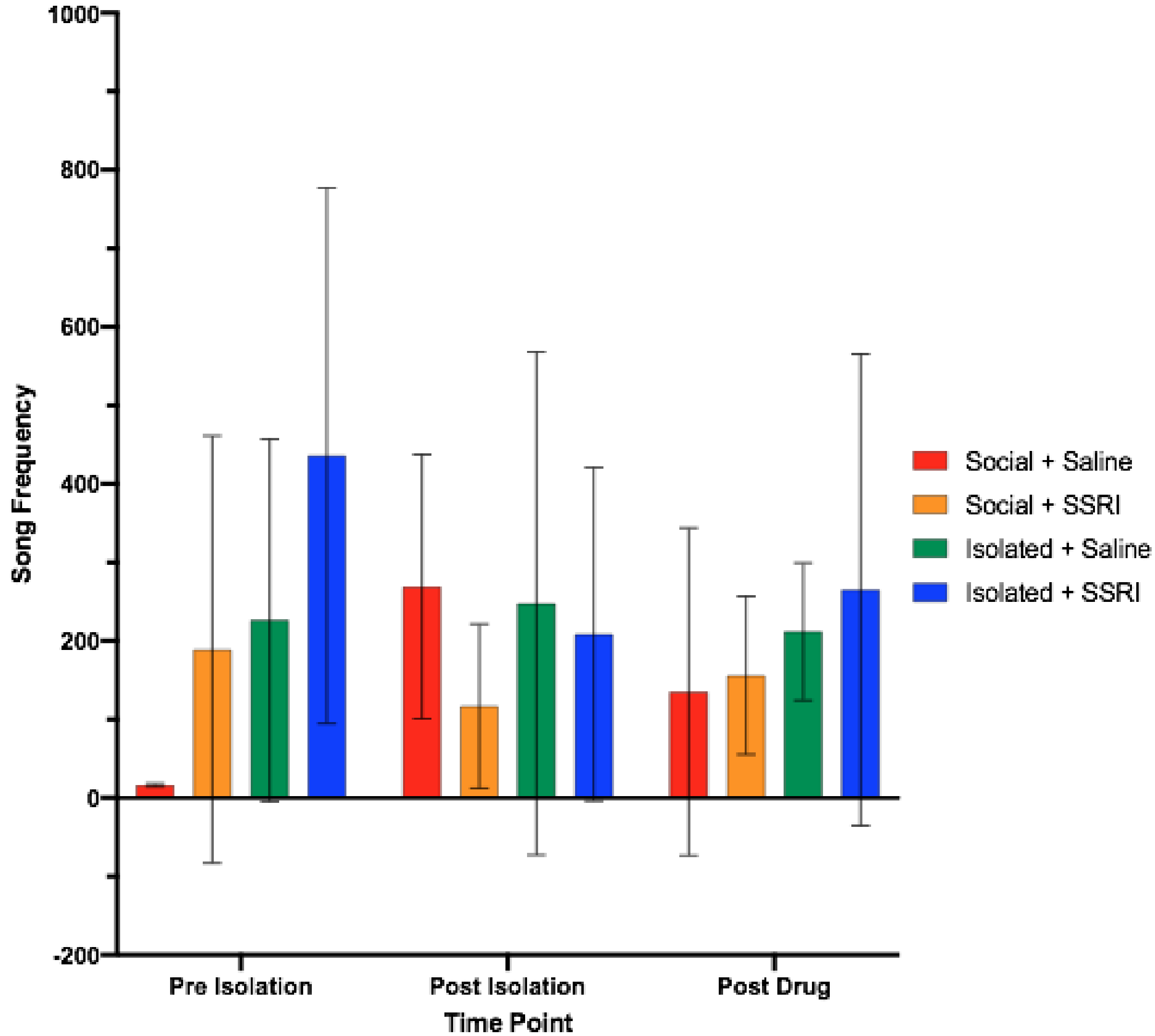

Figure 7. Average song frequency sung by birds in each of the four conditions over the three time points. Error bars refer to standard deviations of each condition at the time point.

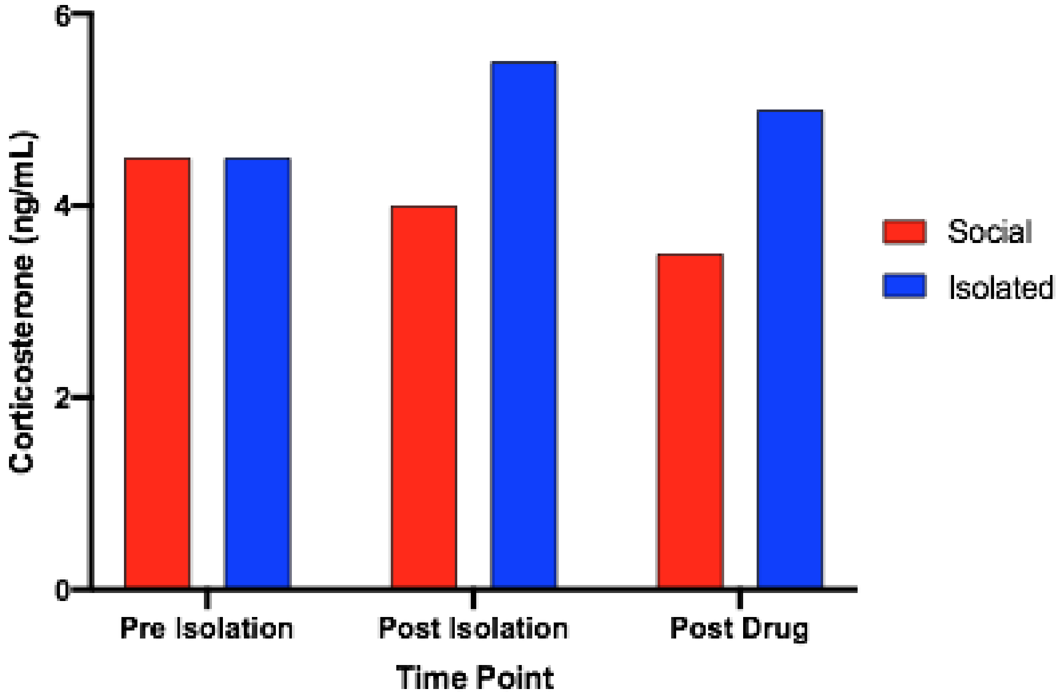

Figure 8. Predicted corticosterone concentrations of the birds in the two environmental conditions over the three time points.

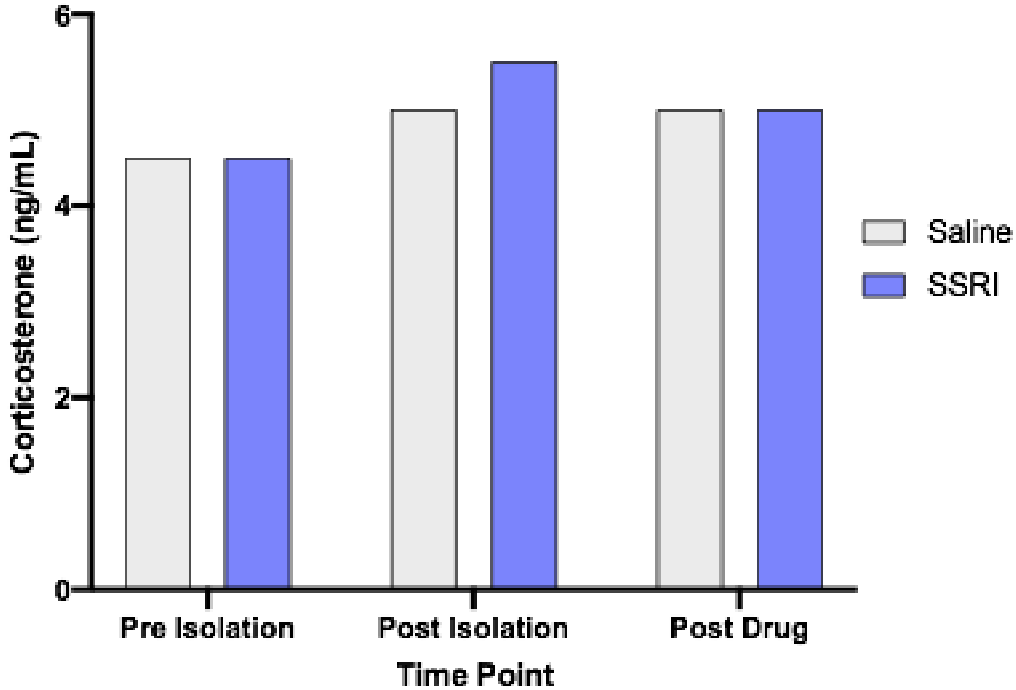

Figure 9. Predicted corticosterone concentrations of the birds in the two drug conditions over the three time points.

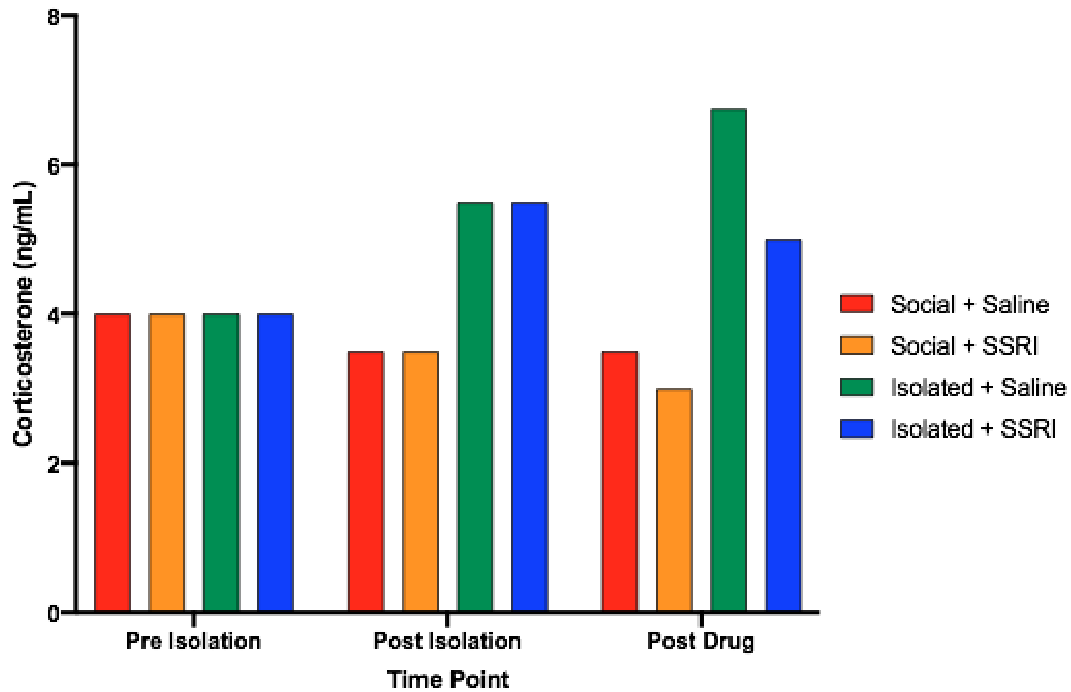

Figure 10. Predicted corticosterone concentrations of the birds in each condition over the three time points.

References

Anacker, C., Zunszain, P. A., Carvalho, L. A., & Pariante, C. M. (2011). The glucocorticoid receptor: pivot of depression and of antidepressant treatment?. *Psychoneuroendocrinology*, *36*(3), 415-425.

Andrus, B. M., Blizinsky, K., Vedell, P. T., Dennis, K., Shukla, P. K., Schaffer, D. J., Radulovic, J., Churchill, G.A., & Redei, E. E. (2012). Gene expression patterns in the hippocampus and amygdala of endogenous depression and chronic stress models. *Molecular psychiatry*, *17*(1), 49.

Arrigo, B. A., & Bullock, J. L. (2008). The psychological effects of solitary confinement on prisoners in supermax units: Reviewing what we know and recommending what should change. *International journal of offender therapy and comparative criminology*, *52*(6), 622-640.

Belmaker, R. H., & Agam, G. (2008). Major depressive disorder. *New England Journal of Medicine*, *358*(1), 55-68.WHO. Depression In: Organization WH, (ed), 2017.

Blendy, J. A. (2006). The role of CREB in depression and antidepressant treatment. *Biological psychiatry*, *59*(12), 1144-1150.

Blundell, J. E., & Halford, J. C. (1998). Serotonin and appetite regulation. *CNS drugs*, *9*(6), 473-495.

Cacioppo, J. T., Hawkley, L. C., & Berntson, G. G. (2003). The anatomy of loneliness. Current directions in psychological science, 12(3), 71-74.

Cacioppo, S., Capitanio, J. P., & Cacioppo, J. T. (2014). Toward a neurology of loneliness. *Psychological bulletin*, *140*(6), 1464.

Cascade, E., Kalali, A. H., & Kennedy, S. H. (2009). Real-world data on SSRI antidepressant side effects. *Psychiatry (Edgmont)*, *6*(2), 16.

Castro, J. E., Varea, E., Márquez, C., Cordero, M. I., Poirier, G., & Sandi, C. (2010). Role of the amygdala in antidepressant effects on hippocampal cell proliferation and survival and on depression-like behavior in the rat. *PloS one*, *5*(1), e8618.

Eysenck, H. J. (1947). Dimensions of personality. London, England: Paul, Trench, Trubner.

Faquih, A. E., Memon, R. I., Hafeez, H., Zeshan, M., & Naveed, S. (2019). A Review of Novel Antidepressants: A Guide for Clinicians. *Cureus*, *11*(3).

Foreyt, J. P., Brunner, R. L., Goodrick, G. K., Cutter, G., Brownell, K. D., & St. Jeor, S. T. (1995). Psychological correlates of weight fluctuation. *International Journal of Eating Disorders*, *17*(3), 263-275.

Gibson-Smith, D., Bot, M., Milaneschi, Y., Twisk, J. W., Visser, M., Brouwer, I. A., & Penninx, B. W. (2016). Major depressive disorder, antidepressant use, and subsequent 2-year weight change patterns in the Netherlands Study of Depression and Anxiety. *The Journal of clinical psychiatry*, *77*(2), e144-51.

Girard, J. M., Cohn, J. F., Mahoor, M. H., Mavadati, S. M., Hammal, Z., & Rosenwald, D. P. (2014). Nonverbal social withdrawal in depression: Evidence from manual and automatic analyses. *Image and vision computing*, *32*(10), 641-647.

Grossman, A. H., D'augelli, A. R., & O'connell, T. S. (2001). Being lesbian, gay, bisexual, and 60 or older in North America. *Journal of Gay & Lesbian Social Services*, *13*(4), 23-40.

Harvey, B. H., & Bouwer, C. D. (2000). Neuropharmacology of paradoxic weight gain with selective serotonin reuptake inhibitors. *Clinical neuropharmacology*, *23*(2), 90-97.

Herman, J. P., McKlveen, J. M., Ghosal, S., Kopp, B., Wulsin, A., Makinson, R., Scheimann, J., & Myers, B. (2016). Regulation of the Hypothalamic-Pituitary-Adrenocortical Stress Response. *Comprehensive Physiology, 6*(2), 603–621. doi:10.1002/cphy.c150015

Hirschfeld, R. M., & Weissman, M. M. (2002). Risk factors for major depression and bipolar disorder.Haney, C. (2006). *Reforming punishment: Psychological limits to the pains of imprisonment*. American Psychological Association.

Hirschfeld, R. M. (1981). Situational depression: validity of the concept. The British Journal of Psychiatry, 139(4), 297-305.

Hobbs, F., & Stoops, N. (2002). *Demographic trends in the 20th century* (Vol. 4). US Census Bureau.

Holt-Lunstad, J., Smith, T. B., & Layton, J. B. (2010). Social relationships and mortality risk: a meta-analytic review. *PLoS medicine*, *7*(7), e1000316.

Holvast, F., Burger, H., de Waal, M. M., van Marwijk, H. W., Comijs, H. C., & Verhaak, P. F. (2015). Loneliness is associated with poor prognosis in late-life depression: Longitudinal analysis of the Netherlands study of depression in older persons. *Journal of affective disorders*, *185*, 1-7.

Jonnakuty, C., & Gragnoli, C. (2008). What do we know about serotonin?. *Journal of cellular physiology*, *217*(2), 301-306.

Keller, J., Gomez, R., Williams, G., Lembke, A., Lazzeroni, L., Murphy Jr, G. M., & Schatzberg, A. F. (2017). HPA axis in major depression: cortisol, clinical symptomatology and genetic variation predict cognition. *Molecular psychiatry*, *22*(4), 527.

Kiecolt-Glaser, J. K., & Glaser, R. (2002). Depression and immune function: central pathways to morbidity and mortality. *Journal of psychosomatic research*, *53*(4), 873-876.

Leonard, B. E. (2010). The concept of depression as a dysfunction of the immune system. In *Depression: From Psychopathology to Pharmacotherapy* (Vol. 27, pp. 53-71). Karger Publisherwin, M. R. (2005). Inflammatory markers and sleep disturbance in major depression. *Psychosomatic medicine*, *67*(2), 187-194.

Lissner, L., Odell, P. M., D'Agostino, R. B., Stokes III, J., Kreger, B. E., Belanger, A. J., & Brownell, K. D. (1991). Variability of body weight and health outcomes in the Framingham population. *New England Journal of Medicine*, *324*(26), 1839-1844.

Maina, G., Albert, U., Salvi, V., & Bogetto, F. (2004). Weight gain during long-term treatment of obsessive-compulsive disorder: a prospective comparison between serotonin reuptake inhibitors. *The Journal of clinical psychiatry*, *65*(10), 1365-1371.

Machado-Vieira, R., Baumann, J., Wheeler-Castillo, C., Latov, D., Henter, I. D., Salvadore, G., & Zarate, C. A. (2010). The timing of antidepressant effects: a comparison of diverse pharmacological and somatic treatments. *Pharmaceuticals*, *3*(1), 19-41.

Matthews, T., Danese, A., Wertz, J., Odgers, C. L., Ambler, A., Moffitt, T. E., & Arseneault, L. (2016). Social isolation, loneliness and depression in young adulthood: a behavioural genetic analysis. *Social psychiatry and psychiatric epidemiology*, *51*(3), 339-348.

McGlashan, E. M., Drummond, S. P. A., & Cain, S. W. (2018). Evening types demonstrate reduced SSRI treatment efficacy. *Chronobiology international*, *35*(8), 1175-1178.

Mondal, A. C., & Fatima, M. (2019). Direct and indirect evidences of BDNF and NGF as key modulators in depression: role of antidepressants treatment. *International Journal of Neuroscience*, *129*(3), 283-296.

Motivala, S. J., Sarfatti, A., Olmos, L., & ICowen, P. J. (2002). Cortisol, serotonin and depression: all stressed out?. *The British Journal of Psychiatry*, *180*(2), 99-100.

Otte, C., Gold, S. M., Penninx, B. W., Pariante, C. M., Etkin, A., Fava, M., Mohr, D. C., & Schatzberg, A. F. (2016). Major depressive disorder. *Nature Reviews Disease Primers*, *2*, 16065.

Porter, R. J., Gallagher, P., Watson, S., & Young, A. H. (2004). Corticosteroid-serotonin interactions in depression: a review of the human evidence. *Psychopharmacology*, *173*(1-2), 1-17.

Pratt, L. A., Brody, D. J., & Gu, Q. (2017). Antidepressant Use among Persons Aged 12 and Over: United States, 2011-2014. NCHS Data Brief. Number 283. *National Center for Health Statistics*.

Primack, B. A., Shensa, A., Escobar-Viera, C. G., Barrett, E. L., Sidani, J. E., Colditz, J. B., & James, A. E. (2017). Use of multiple social media platforms and symptoms of depression and anxiety: A nationally-representative study among US young adults. *Computers in human behavior*, *69*, 1-9.

Roelofs, K., Hagenaars, M. A., & Stins, J. (2010). Facing freeze: social threat induces bodily freeze in humans. *Psychological Science*, *21*(11), 1575-1581.

Ronaldson, A., Carvalho, L. A., Kostich, K., Lazzarino, A. I., Urbanova, L., & Steptoe, A. (2018). The effects of six-day SSRI administration on diurnal cortisol secretion in healthy volunteers. *Psychopharmacology*, *235*(12), 3415-3422.

Rubio-Guerra, A. F., Rodriguez-Lopez, L., Vargas-Ayala, G., Huerta-Ramirez, S., Serna, D. C., & Lozano-Nuevo, J. J. (2013). Depression increases the risk for uncontrolled hypertension. *Experimental & Clinical Cardiology*, *18*(1), 10.

Sargin, D., Oliver, D. K., & Lambe, E. K. (2016). Chronic social isolation reduces 5-HT neuronal activity via upregulated SK3 calcium-activated potassium channels. *Elife*, *5*, e21416.

Schimelpfening, N. (2019). Differences of Endogenous and Exogenous Depression. Retrieved from https://www.verywellmind.com/what-is-endogenous-depression-1067283.

Singh, A., & Misra, N. (2009). Loneliness, depression and sociability in old age. *Industrial psychiatry journal*, *18*(1), 51.

Stephens, M. A. C., & Wand, G. (2012). Stress and the HPA axi: Role of glucocorticoids in alcohol dependence. *Alcohol research: current reviews*.

Vythilingam, M., Vermetten, E., Anderson, G. M., Luckenbaugh, D., Anderson, E. R., Snow, J., Staib, L.H., & Bremner, J. D. (2004). Hippocampal volume, memory, and cortisol status in major depressive disorder: effects of treatment. *Biological psychiatry*, *56*(2), 101-112.

Wannamethee, S. G., Shaper, A. G., & Walker, M. (2002). Weight change, weight fluctuation, and mortality. *Archives of internal medicine*, *162*(22), 2575-2580.

Winerman, L. (2017). By the numbers: Antidepressant use on the rise. American Psychology Association. *48*(10), 120.

World Health Organization (2020). Depression.

Yang, L., Zhao, Y., Wang, Y., Liu, L., Zhang, X., Li, B., & Cui, R. (2015). The effects of psychological stress on depression. *Current neuropharmacology*, *13*(4), 494-504.