Tamoxifen Influences on Behavior and Mood in Female Rats

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TAMOXIFEN AND BEHAVIOR

Abstract

Tamoxifen is a selective estrogen receptor modulator (SERM) used in the treatment of hormone receptor-positive breast cancer in pre-menopausal and post-menopausal women. The purpose of this study was to analyze the effects of long-term tamoxifen treatment on anxiety and depressive behaviors in female rats. Female rats were randomly assigned to receive tamoxifen treatment through medicated food pellets or unmedicated normal rat chow for 10-13 weeks. It was hypothesized that tamoxifen rats would exhibit increased anxiety and depressive behaviors in the elevated plus maze and open field test as a result of the decrease in estrogen activity in the brain because of tamoxifen treatment. Increased anxiety behavior in the elevated plus maze would be associated with increased time spent in the enclosed arms of the maze and increased entries into the enclosed arms versus the open arms. In the open field test increased anxiety behavior would be associated with a lower number of central crossings of the open field and increased time spent on the outer parts of the open field near the walls and corners. Because of the COVID-19 pandemic, the behavioral data from the study was unavailable for analysis at this time. Analysis of trends in weight data for rats in the study found that tamoxifen treatment was significantly associated with differences in weight change compared to control rats during the study. Tamoxifen treatment was associated with an initial decrease in body weight and a lower overall end weight relative to control rats. Future studies should continue the work of this study by analyzing the behavioral data of this study and designing other experiments to also examine cognitive effects associated with long-term tamoxifen treatment. Future immunocytochemistry analysis of hippocampal circuitry is also important in understanding the behavioral and cognitive effects of long-term tamoxifen treatment.

Keywords: tamoxifen, estrogen, anxiety, elevated plus maze, open field test
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Tamoxifen Influences on Behavior and Mood in Female Rats

Approximately 1 out of every 8 women in the United States will develop breast cancer over their lifetime, and approximately 40,000 women die as a result of breast cancer each year in the United States (Breast Cancer Statistics | CDC, 2019; Siegel et al., 2019). Surgery, chemotherapy, and hormonal therapy are the most common treatments for breast cancer. Hormone therapy is a significant part of breast cancer treatment because it is used to shrink tumors before surgery and prevent tumor reoccurrence following surgery. Hormonal therapy is used to treat hormone-receptor-positive breast cancer, a type of cancer in which cancer cells use natural hormones in the body, like estrogen, to grow and develop (Drăgănescu & Carmocan, 2017). Approximately two-thirds of women with breast cancer have hormone-receptor-positive breast cancer, meaning they will undergo endocrine therapy as treatment. Tamoxifen is the most widely used drug for adjuvant hormone therapy to treat hormone-receptor-positive breast cancer and has been prescribed since its approval by the U.S. Food and Drug Administration in 1977 (Osborne, 1998).

Hormone therapies, like tamoxifen, typically are prescribed for extensive treatment windows that can last 10 years or longer in some cases (Davies et al., 2013). These therapies that affect hormone levels for an extended period of time may have significant effects throughout the body. Tamoxifen is administered to modulate activity at the estrogen receptors of breast cancer cells, but can impact estrogen activity in other areas of the body (Lichtenfels et al., 2017). Estrogen is a neuroprotective hormone that affects the brain and behavior throughout the lifespan of women. Recent research has suggested that decreasing levels of estrogen in postmenopausal women is a risk factor for neurodegeneration and the development of Alzheimer’s, exhibiting the importance of estrogen in the mental health of women throughout life, as women are two-thirds
more likely to develop Alzheimer’s during their lifetime (Janicki & Schupf, 2010; Viña & Lloret, 2010).

Based on the widespread use of tamoxifen as an adjuvant hormone therapy for the treatment of breast cancer and the important role of estrogen in the mental health of women, this study intends to investigate the effects of long-term tamoxifen treatment on behavioral measures in Long-Evans Hooded female rats.

**Tamoxifen**

*Mechanism of Action*

Tamoxifen is a nonsteroidal triphenylethylene derivative that acts as a selective estrogen receptor modulator (SERM) used in the treatment of both pre- and post-menopausal women diagnosed with hormone-receptor-positive breast cancer (Shagufta & Ahmad, 2018). The mechanism of action of tamoxifen is multi-dimensional as the drug acts as an estrogen antagonist in hormone-receptor-positive breast cancer cells, a partial estrogen agonist in endometrial cells, and an estrogen agonist in bone cells (Drăgănescu & Carmocan, 2017). The central antagonistic mechanism of tamoxifen is accomplished by its binding to estrogen receptors to block the binding of estrogen in mammary epithelial cells (Sporn & Lippman, 2003). Tamoxifen is able to block estrogen binding due to its similar structure and chemical makeup relative to estrogen as its binding at estrogen receptors induces changes in the three-dimensional conformation of the receptors (Goodsell, 2002). Tamoxifen is typically administered at a dose of 20 mg daily, with a half-life following an initial dose of approximately 9 to 12 hours, and a half-life following chronic dosing of around 7 days (Fabian et al., 1981).

In addition to its main mechanism of action in breast cancer cells, tamoxifen can also exert different effects throughout the body through various mechanisms. Notably, tamoxifen can
transverse the blood-brain barrier and consequently has the ability to interact with estrogen
receptors present throughout the brain (Rotheneichner et al., 2017). With the capacity to
transverse the blood-brain barrier and affect estrogen activity in the brain, tamoxifen can alter the
brain and impact behavior.

Tamoxifen and Behavior

Research has found that treatment with tamoxifen for ten years instead of only five years
is associated with less breast cancer recurrence and lower mortality rates for patients. The
international Adjuvant Tamoxifen: Longer Against Shorter (ATLAS) trial randomly assigned
women with breast cancer to either continue tamoxifen for ten years or stop at five years (Davies
et al., n.d.). Tamoxifen treatment for ten years instead of only five years was associated with
reduced recurrences and mortality in participants with ER-positive breast cancer (Davies et al.,
2013). Even with studies supporting the extended treatment window of tamoxifen as an adjuvant
therapy for breast cancer, there is a surprising lack of research investigating the effects of long-
term tamoxifen treatment. Tamoxifen tends to elicit symptoms similar to those of menopause,
postpartum depression, and premenstrual syndrome as a result of the effects of tamoxifen on
estrogen activity (Moon et al., 2017). Given the use of tamoxifen over extended time periods and
the ability of tamoxifen to transverse the blood-brain barrier, it is important to investigate the
possible effects of tamoxifen on the brain and behavior.

Any drug that has a mechanism of action that affects hormones has the potential for
wide-ranging physiological effects. Even though tamoxifen has a treatment window of
approximately 10 years as a drug therapy for breast cancer, there is a surprising lack of research
on its potential effects on the brain and behavior. Research has implied possible clinical
associations between anxiety and tamoxifen treatment in breast cancer patients (Cameron et al.,
A previous study found treatment with tamoxifen to be associated with increased anxiety behaviors in postmenopausal women with breast cancer in remission (Cameron et al., 1998). Additionally, breast cancer patients have presented with acute depressive symptoms in response to tamoxifen treatment that required treatment with venlafaxine, a selective serotonin and norepinephrine reuptake inhibitor that treats depression (Bourque et al., 2009).

Behavioral effects of tamoxifen treatment are important to understand given the length of time it is used for treatment and the circumstances surrounding its use. Tamoxifen, since its approval by the FDA in 1977, has been the most widely prescribed drug for adjuvant hormone therapy in the treatment of breast cancer. In addition to its use to treat breast cancer in premenopausal and postmenopausal women, it is also used preventatively in healthy women with significantly increased risk of developing breast cancer. Patients who have been diagnosed with breast cancer and are being treated with tamoxifen already face the stress and anxiety associated with their diagnosis. If tamoxifen is associated with increased anxiety and stress behaviors, breast cancer patients being treated with tamoxifen would face increased burden to their psychological well-being and health that could negatively impact their disease progression and response to treatments.

**Tamoxifen as an Estrogen Antagonist: Estrogen and the Brain**

The behavioral implications observed as a result of tamoxifen treatment are assumed to be a result of their antagonistic effects on estrogen levels in the brain. Along with progesterone, estrogen is one of the sex hormones in females. Produced by the ovaries, estrogen regulates the menstrual cycle of women and is necessary for childbearing while mediating the development of women throughout the lifespan. Once menopause occurs, the ovaries cease the production of estrogen and estrogen levels in the body drop. Changes in estrogen levels throughout the lifespan
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and especially following menopause have important effects on health. Decreasing estrogen levels can affect bone health and can contribute to the development of osteoporosis while also affecting the brain and cognition (Morrison & Tweedy, 2000; Pettersson & Gustafsson, 2001). Through its antagonistic effect at estrogen receptors, tamoxifen can decrease estrogen activity in the brain, and impact behavior and mood. Based on the mechanism of action of tamoxifen, it is believed that some of its unintended side effects result from its effect on estrogen activity since treatment with tamoxifen elicits symptoms associated with menopause, postpartum depression, and premenstrual syndrome, all periods in which significant changes in estrogen levels occur (Eberling et al., 2004).

During menopause the ovaries stop producing estrogen causing the levels of the hormone to decrease. Mood swings, depression, anxiety, and cognitive deficits have been associated with menopause as a result of the decrease in estrogen levels. Natural and surgical menopause as well as antiestrogen therapy to treat breast cancer patients are associated with increased presentation of depression and a lack of efficacy of antidepressant medications, especially in aging women (Morrison & Tweedy, 2000). A randomized, double-blinded, placebo-controlled clinical trial supported the effects of decreased estrogen levels on behavior and mood as estrogen therapy improved mood in postmenopausal women (Gleason et al., 2015). Estrogen replacement therapy (ERT) benefits mood in postmenopausal women through its effects on neurotransmitters, including serotonin and norepinephrine (Halbreich, 1997). There is limited research on the mechanism of action of tamoxifen in the central nervous system and varied evidence of the behavioral and mood implications that are associated with treatment using this selective estrogen receptor modulator. Clinical reports indicate that tamoxifen increases depressive symptoms in
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breast cancer patients suggesting it acts as an estrogen antagonist in the central nervous system (Henry et al., 2008; Morrison & Tweedy, 2000; Thompson et al., 1999).

**Animal Models and Tamoxifen**

Tamoxifen effects on estrogen in the brain could significantly impact behavior in women with breast cancer and subsequently affect health outcomes of patients. One study investigated the effects of tamoxifen treatment for 4 weeks on ovarian hormones with respect to anxiety and depressive behaviors in female rats. Two treatments in the study that were analyzed were ovariectomy (removal of the estrogen-producing ovaries) and tamoxifen treatment. Decreases in estrogen levels resulting from ovariectomy and tamoxifen treatment were associated with increased anxiety and depressive behaviors (Azizi-Malekabadi et al., 2015). Increased anxiety and depressive behaviors manifested in increased immobility times during the forced swimming test, decreased central crossings in the open field test, and decreased entries into the open arms of the elevated plus maze (Azizi-Malekabadi et al., 2015). This study utilized several behavioral paradigms of anxiety and depression commonly used in rodent studies. These tests will be described in detail in future sections. Further support for the association between increased anxiety and tamoxifen administration in female rats was reported by Zimmerberg and Farley (1993). Rats receiving tamoxifen injections or ovariectomy exhibited increased anxiety behaviors. Specifically, female rats who received either tamoxifen injections or ovariectomy spent less time on the open arms of the elevated plus-maze than controls (Zimmerberg & Farley, 1993). These studies citing the behavioral effects of tamoxifen treatment add to the previous literature on the behavioral effects of decreased estrogen levels corresponding to depressive behavior manifested in decreased exploratory behavior in female rats following ovariectomy (Djiogue et al., 2018).
Based on previous literature citing the behavioral effects of tamoxifen treatment that parallel the effects of decreased estrogen levels, it is important to further investigate the behavioral impacts of long-term tamoxifen treatment. The behavioral effects of tamoxifen treatment have significant clinical relevance and significance to breast cancer patients undergoing treatment with tamoxifen. Large-scale clinical studies analyzing the behavioral effects of long-term tamoxifen treatment are difficult because they rely on relatively subjective self-report measures of behavior and are susceptible to varying confounds including individual differences, varying levels of drug adherence, and ethical limitations. As summarized above, animal models investigating the effects of tamoxifen on anxiety measures have administered this drug for a relatively short period of time and thus do not model the clinical reality of tamoxifen treatment.

**Long-Term Tamoxifen Treatment Effects on Behavior**

As current protocols in treating breast cancer suggest a tamoxifen regimen of 10 years or longer, and the presence of a strong association between decreased estrogen levels and anxiety and depression, there is a need to better understand the impact of this SERM on the mental health of women. To date there are relatively few studies examining the specific effects that tamoxifen can have following long-term treatment. Given the behavioral effects associated with tamoxifen in several studies and its mechanism of action as a selective estrogen receptor modulator, it is important to investigate the effects of long-term tamoxifen treatment on the brain and behavior.

**The Present Study**

The primary objective of this study was to determine whether long-term administration of tamoxifen over the course of 12 weeks in female rats was associated with behavioral effects compared to rats who did not undergo tamoxifen treatment. It was hypothesized that behavior of
female rats would be negatively affected by long-term tamoxifen treatment based on the antagonistic effects of tamoxifen on estrogen receptors and the role estrogen plays in the brain as well as its association with behavior.

Choice of Species. A rodent model was the optimal method for conducting a controlled experiment on long-term tamoxifen treatment. Rats were chosen as the rodent model for use in this study for a few reasons. First, it was believed that the feeding behavior of rats would be more favorable to the administration of tamoxifen through their diets (Nutrition, 1995). Based on the lifespan of rats, it was determined that 12 weeks in middle-aged rats effectively modeled the long-term treatment of tamoxifen that would be observed in a human clinical setting (Sengupta, 2013). Rats were also used because of the well-established behavioral paradigms available for assessing outcome measures for the study.

Route of Administration. Because the goal of the study was to analyze long-term tamoxifen treatment, the administration of tamoxifen in the food pellets of the rats was the most feasible route of administration. The use of the oral gavage technique, delivery of liquid directly into the stomach of the animal using a gavage needle, and injection were other routes of administration of tamoxifen used in past studies but were infeasible here because of the stress these techniques would bring to the rat during daily treatment over a long-term treatment window.

Behavioral Assessment. Behavioral tests were used to assess anxiety and depressive behaviors in response to long-term tamoxifen treatment. Previous studies identified differences in behavior that result from differences in estrogen levels following ovariectomy or tamoxifen treatment using the elevated plus maze and open field test. In these studies, decreased estrogen
levels were associated with increased-anxiety behavior and less exploratory behavior exhibited during the tests (Azizi-Malekabadi et al., 2015; Djiove et al., 2018; Walf & Frye, 2007).

The elevated plus maze is a validated behavioral paradigm for assessing anxiety. The maze consists of an elevated platform with two vertical open arms and two horizontal enclosed arms. The elevated plus maze has been validated through behavioral, physiological, and pharmacological assessments as increased time spent by rats in the enclosed arms correlated significantly with increased anxiety. Rats made significantly fewer entries into the open arms of the maze and activity in the open arms of the maze was associated with increased stress levels assessed using plasma hormone levels (Pellow et al., 1985). Also, anxiolytic pharmacological compounds used to treat anxiety clinically were associated with increased numbers of entries to the open arms of the maze and increased overall time spent in the open arms of the maze by the rats (Pellow et al., 1985).

In the elevated plus maze, anxiety behavior is assessed using several measures including the ratio of time spent in the enclosed versus the open arms for each rat and the number of entries made into the closed arms versus the open arms of the maze for each rat. The behavioral paradigm of the elevated plus maze is based on the approach-avoidance conflict in rodents that posits that rodents have an innate tendency to explore novel environments while preferring enclosed and darker spaces that would confer safety from threats in the environment and predators (Bailey & Crawley, 2009). Increased time spent in the open arms of the maze is associated with decreased anxiety behavior while increased time spent in the enclosed arms of the maze is associated with increased anxiety behavior (Walf & Frye, 2007).

The open field test is another behavioral paradigm that is used to assess anxiety and depressive behaviors. The open field typically consists of a square open space enclosed by 4
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walls where the animals can explore. The test assesses exploratory and locomotor behaviors in rats with activity level being assessed during the test quantitatively by tracking central crossings, rearing, and time spent moving (Valvassori et al., 2017). Additionally, behaviors such as defecation or urination can be used as additional measures of anxiety behavior in rats (Seibenhener & Wooten, 2015).

In the open field test, anxiety and depressive behavior is assessed using several measures including amount of time spent on the outer parts of the open field next to the walls and in the corners versus amount of time spent in the center of the open field, as well as the overall movement around the open field, average velocity, and number of central and peripheral crossings. Increased movement patterns and increased time spent in the open space of the field are typically indicative of decreased anxiety and depressive behavior (Seibenhener & Wooten, 2015). Like the elevated plus maze, the open field test is based on the tendency for rodents to explore novel environments allowing for assessment of locomotor activity and observation of different forms of possible behavioral indices of anxiety. It is expected that rodents with increased anxiety would have a significant aversion to the central part of a novel environment, like the open field, initially because it offers little protection from environmental dangers. Habituation to the open field over time, however, occurs as animals become use to the open field and more comfortable in the environment (Bailey & Crawley, 2009). Both the open field test and elevated plus maze were used to assess behavioral outcome measures in the present study to observe the effects of long-term tamoxifen treatment in female rats.

Summary of Current Study and Hypotheses. The purpose of this study was to analyze the effects of long-term tamoxifen treatment on anxiety and depressive behaviors in female rats. Female rats received either tamoxifen treatment through medicated food pellets or unmedicated
normal rat chow for 12 weeks. It was hypothesized that tamoxifen rats would exhibit increased anxiety and depressive behaviors in the elevated plus maze and open field test as a result of the decrease in estrogen activity in the brain because of tamoxifen treatment. Increased anxiety behavior in the elevated plus maze would be associated with increased time spent in the enclosed arms of the maze and increased entries into the enclosed arms versus the open arms. In the open field test increased anxiety behavior would be associated with a lower number of central crossings of the open field and increased time spent on the outer parts of the open field near the walls and corners.

**Method**

All procedures met the recommendations of the Guide for Care and Use of Laboratory Animals of the National Institutes of Health. Protocol approval was obtained by Haverford College’s Animal Care and Use Committee (Protocol Number: 101619).

**Animals**

36 total female Long-Evans Hooded rats, 3-4 months of age and with a weight of 250 g at the beginning of the experiment, were used in the study. Rats were obtained from Charles River Laboratories (Charles River, Kingston, PA) and housed in pairs in standard laboratory housing cages throughout the course of the study. Cages contained standard TekFresh (Teklad 7099) cellulose low-dust rat bedding, a tube, and a chewing block for the rats to explore. Cages were housed in a 12-hour light-dark cycle with access to food and water *ad libitum*. The 250 g rat was an effective model because it is a middle-aged rat (3-4 months of age based on information from Charles River), representing the typical demographic of patient who would receive tamoxifen in a clinical setting. The experimental procedures were approved in accordance with IACUC approval.
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Experimental Groups

In this study, cages were randomly assigned to receive long-term tamoxifen treatment via medicated food pellets obtained from Envigo (Envigo.com) or unmedicated food pellets. The beginning of tamoxifen treatment and initiation of the experimental protocol was staggered into two different cohorts to ensure the feasibility of the protocol and to stagger the behavioral testing at the conclusion of the protocol.

**Cohort 1.** 8 rats (4 cages) were assigned to the tamoxifen treatment group and 8 rats (4 cages) assigned to the control group for Cohort 1 of the study (see Table 1). Rats assigned to Cohort 1 began the treatment protocol 3 weeks after arrival to Haverford College.

**Cohort 2.** 5 weeks following initiation of the experimental protocol in Cohort 1, 12 of the remaining rats (6 cages) were randomly assigned to begin tamoxifen treatment and 8 rats (4 cages) were assigned to remain on unmedicated food pellets for Cohort 2 of the study (see Table 1).

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<th>Tamoxifen Treatment</th>
<th>Unmedicated Food Treatment</th>
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<tr>
<td>Cohort 1</td>
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<td>Cohort 2</td>
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Table 1. Overview of rats assigned to the two treatment groups for Cohort 1 and Cohort 2.

Procedure

Tamoxifen administration was initiated 3 weeks after arrival for rats assigned to the tamoxifen treatment group in Cohort 1 and 8 weeks after arrival for rats assigned to the tamoxifen treatment group in Cohort 2. Tamoxifen administration for Cohort 1 lasted 13 weeks. Due to the COVID-19 public health emergency, the protocol for Cohort 2 was terminated approximately 10 weeks following initiation. 10-13 weeks of tamoxifen treatment enabled the
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observation of the long-term effects of tamoxifen on behavior and mood. Following the conclusion of tamoxifen treatment in each cohort, behavioral testing was conducted. Behavioral tests were used to assess anxiety and depressive behaviors. Following the conclusion of behavioral testing, the animals were sacrificed, and their brains removed for future analysis.

**Tamoxifen Administration**

Based on the advisement of the nutritionist from Envigo and the weight and sex of the rats used in the study, it was determined that the rats should eat approximately 13 g of food per day. Food and water were checked and refilled daily. It was determined that medicated food pellets were the optimal mode of delivery for the tamoxifen treatment. Tamoxifen was administered through food pellets containing the drug purchased from Envigo (Envigo.com). The optimal dosage was contained in the food pellets used for tamoxifen treatment and was similar relative to the typical dosage of tamoxifen prescribed to premenopausal women as an adjuvant therapy for breast cancer treatment, approximately 20 mg (*Tamoxifen Dosage Guide with Precautions*, n.d.). The dosage included in the food pellet was determined using a standard conversion to identify correct dosages of drugs for use in animal models based on the ratio of the body weight of the animal model relative to human body weight (Reagan-Shaw et al., 2008).

Based on previous literature it was expected that tamoxifen treatment would be associated with subsequent weight loss resulting from metabolic changes in the rats receiving treatment (Lampert et al., 2013; Liu et al., 2018).

**Tamoxifen Safety Precautions.** Because tamoxifen is a carcinogen necessary precautions were taken to ensure the safety of the researchers. This included keeping containers holding tamoxifen feed secured, wearing personal protective gear including gloves, mask,
Health Monitoring and Assessment. During the experimental protocol, rats were observed daily to track any significant health or behavioral changes. In addition, rats were weighed regularly to ensure each rat remained within 20% of their pre-treatment body weight, with bedding and cages changed and cleaned weekly. Due to the nature of the weight loss that was expected in rats undergoing tamoxifen treatment, tamoxifen rats were weighed more regularly than control rats. The BCS scale was implemented to the experimental protocol for Cohort 2 in the study. In consultation with a licensed veterinarian, it was determined that the BCS scale could be used to assess health in addition to monitoring the body weights of the rats (Hickman & Swan, 2010). All rats maintained well-conditioned scores on the BCS scale of 3 or greater (Appendix B). BCS was assessed weekly by a blinded observer during cage changes. If the BCS score dropped below 3, rats were removed from the protocol and resumed feeding with normal unmedicated rat chow.

Cohort 1. Tamoxifen treatment was initiated in 8 rats randomly assigned to the experimental group with normal food chow in the rats’ cage hopper replaced with medicated tamoxifen food pellets. 8 control rats were matched to the experimental rats receiving tamoxifen treatment in Cohort 1 and remained on a diet consisting of unmedicated rat chow. Following initiation of tamoxifen treatment, several rats exhibited weight loss greater than 15% of their body weight. To mitigate weight loss observed in rats receiving tamoxifen treatment, a food mash began to be used for daily feeding. This mash was made by placing 10-12 tamoxifen food pellets into a plastic mixing bowl to ensure that each rat received approximately 2 pellets. After being added to the plastic mixing bowl, the pellets were soaked for 30-45 minutes. Mash was
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then mixed with roughly a ½ spoonful of recovery diet gel (Clear H20, Portland, ME, USA). The mash was then separated evenly and placed on folded wet paper towels. Two paper towels with mash were placed into each cage so that each rat had a serving of food mash. In addition to the mash, two tamoxifen pellets were also placed in each cage. Following implementation of the food mash for feeding in the tamoxifen rats, the tamoxifen rats were weighed every two days with control rats weighed once a week. To prevent the introduction of any confounds to behavior, control rats began to be handled each time tamoxifen rats were weighed. This was to ensure that both groups of rats had similar exposure to handling by researchers by the start of behavioral testing.

*Cohort 2.* 5 weeks after the initiation of the experimental protocol in Cohort 1, 12 of the remaining rats began to receive tamoxifen treatment through medicated food pellets. As in Cohort 1, these rats were matched with 8 rats who continued to receive normal unmedicated rat chow in the control group. Based on the experience with the experimental protocol in Cohort 1 with weight loss observed in the tamoxifen rats ranging from 7-21% of their initial body weight, the protocol for tamoxifen initiation in Cohort 2 was amended. In Cohort 2 tamoxifen treatment was initiated in rats by a mash mixture containing a blend of medicated and unmedicated pellets. The mash was prepared by soaking 10 medicated and 10 unmedicated food pellets in separate plastic mixing bowl for 30-45 minutes. A 50/50 mash mixture was then created in a small plastic mixing bowl and ½ spoonful of recovery diet gel (Clear H20, Portland, ME, USA) was added. Once the mash was prepared, it was evenly separated onto folded wet paper towels and placed in the cages of the tamoxifen rats. Over the initial week of tamoxifen treatment, the amount of unmedicated pellets included in the mash was gradually decreased so that after the first week of treatment only medicated tamoxifen pellets were included in the mash given to the tamoxifen
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rats. Tamoxifen rats were weighed bi-weekly to ensure maintenance of body weight. Another important amendment introduced in Cohort 2 was the feeding of a small amount of mash to control rats. To eliminate any possible confounding factors to behavior associated with opening the rats’ cages each day and providing them with prepared food mash with diet gel, food mash made of unmedicated pellets and a small amount of diet gel was made daily and given to the control group rats. After feeding rats with mash, two of their respective food pellets were left in their cages for additional food.

In addition to the amendments to the route of administration of the food in Cohort 2 for both the experimental tamoxifen group and the unmedicated control group, the BCS scale was also used to monitor health in conjunction with body weight monitoring. The BCS scale was important to health monitoring because introduction of tamoxifen treatment in older rats with greater basal weights at the beginning of treatment led rats to lose a greater percentage of their body weight than the rats in Cohort 1, exceeding the 20% percent change in body weight mark established in the experimental protocol for health monitoring. BCS was assessed weekly by blinded observers and all rats maintained a BCS score of 3 or greater with a BCS score of less than 3 warranting removal from the study (Appendix B). Based on assessments conducted by a veterinarian and laboratory assistants with experience using the BCS scale, it was determined that all experimental rats were at a level of 3 or higher meaning that they were well-conditioned while most control group rats were over-conditioned at a level of 4 on the scale. The BCS scale was used to ensure and monitor the safety of the rats in the study throughout the protocol.

Behavioral Testing

Following tamoxifen treatment, the rats underwent behavioral tests to assess indices of anxiety and mood. All rats underwent behavioral testing in randomized groups. Cohort 1 began
behavioral testing 5 days after chronic tamoxifen administration ended and Cohort 2 began behavioral testing 3 days after chronic tamoxifen administration ended. The behavioral protocols employed were to measure differences between groups in anxiety utilizing the elevated plus maze and open field test. Prior to conducting behavioral testing, a random number generator was used to assign and counterbalance the order for testing by randomly generating the order of rats for testing and randomly assigning whether rats were tested in the elevated plus maze or open field test first. Notecards were made with blinded identification numbers corresponding to the rats undergoing testing to ensure the researchers were blinded to the identity of the rats undergoing testing. Once the order for testing was established, clean testing cages were arranged with new cellulose bedding. The rats were transferred from their housing cages to the newly prepared testing cages and their hoppers and tubes transferred as well as their identification cards. The testing cages were placed on a cart and moved to outside the testing room and allowed to acclimate for 30 minutes prior to the beginning of testing. Prior to the beginning of testing, the video cameras and video tracking software were setup. After the 30 minutes for acclimatization were finished, the cages of the rats were brought into the testing room when their number came up based on the established order for testing. Between each testing period, the elevated plus maze and open field test were cleaned using 100% EtOH. Tests were conducted in the same rooms at the same time each day of testing.

**Elevated Plus Maze.** The elevated plus maze is an apparatus consisting of two horizontal enclosed arms, 51 x 11.5 cm, and two vertical open arms, 51 x 11.5 x 39.5 cm, with an open roof, arranged so that the two open arms are opposite each other. In the center of the apparatus, where the arms meet, there is a 10 cm x 11 cm space large enough for the animal to stand. The maze is elevated to a height of 73 cm off the ground. Rats were placed in the open space at the
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junction of the four arms of the maze facing the back open arm and their behavior was observed and simultaneously video recorded for 5 minutes. Two blinded observers were present to observe and monitor the behavior of the rat.

Locomotor activity of the rats was recorded by a digital video camera (Canon Vixia HF R600) suspended above the apparatus for a 5-minute period. The videos were subsequently stored for analysis using the Noldus Etho-Vision-XT system (Noldus Information Technology, the Netherlands, v.14). This system uses a pixel-based detection algorithm to localize the animal within the apparatus and track their movement. Video-recordings were stored for analysis of anxiety-like behavior including amount of time spent in the open arms, amount of time spent in the closed arms, difference between time spent in the closed and open arms as well as non-specific locomotor behavior including distance traveled and average velocity.

**Open Field Test.** The second behavioral test that was used to test anxiety behavior and behaviors suggestive of lethargy and depression was the open field test. The open field utilized a square space with dimensions 50 cm x 50 cm enclosed by walls measuring 38 cm in height. In the open field test, each rat was placed in the center of the open field facing the back wall. The behavior of each rat was recorded using a digital video camera (Canon Vixia HF R600) on a camera stand above the open field and uploaded to Noldus Etho-Vision-XT system (Noldus Information Technology, the Netherlands, v.14) to assess overall movement and other behaviors of the rats during the 5-minute testing period.

**Blood Draw and Perfusion**

**Blood Draw.** Important for the protocol in this study was ensuring significant physiological dosage of tamoxifen in the rats receiving long-term tamoxifen treatment. Tamoxifen metabolites are present in the blood during chronic tamoxifen treatment (Lien et al.,
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1991). To this end, tamoxifen levels and their active metabolites were assessed. After the animals were deeply anesthetized and the internal organs were exposed through the initial incision, 2-3 mL of blood were extracted from the caudal vena cava using an 18-gauge needle. Blood was obtained from all rats treated with tamoxifen during the study and a random sample of control rats for comparison. Following blood draw, the blood was quickly inserted into heparin tubes for preparation of assay.

**Intracardial-Perfusion.** Sodium pentobarbital with phenytoin (Beuthanasia-D, 22 mg/100 g body weight) was used to sacrifice the animals in the study. It was a quick and safe mode of inducing an anesthetic state prior to perfusion. First, rats were anesthetized using 5% isoflurane in an anesthesia chamber. Once there was an absence of an eye blink reflex and no response to a toe pinch, perfusion was started in the rats. A second administration of Beuthanasia-D (0.05-0.1 CCs) was administered when necessary to achieve complete anesthesia. Next, an incision across the abdomen was made through the skin and muscle layers with scissors, making sure to avoid damaging the liver and other organs of the animal. Scissors were then used to cut through the ribcage to expose the heart of the animal and the diaphragm was cut on the left and right side to stop breathing. Next, an 18G needle that was attached to a perfusion pump was placed in the left ventricle of the heart and the right atrium was cut to create an open circuit so that blood and perfusion solutions could drain from the animal. Each animal was perfused intracardially using approximately 50 mL of 25 mM phosphate buffered saline followed by approximately 500 mL of 4% paraformaldehyde in 25 mM phosphate buffered saline. At the conclusion, the animal’s head was removed, and the brain harvested for subsequent tissue processing and immunocytochemistry.
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Statistics

Statistical Analysis. Weight change was analyzed statistically using two time points for the cohorts, the initial weight at the start of the experimental protocol and the final weight prior to the beginning of behavioral testing. Two independent samples t-tests were run for both cohorts comparing the mean weight change between the tamoxifen treatment rats and the control rats. Although a repeated measures analysis of the weight data may have been more appropriate analyzing weight change over the course of the protocol, independent samples t-tests were used for the exploratory analysis of weight change conducted in this study.

Results

Because of unexpected and sudden changes to access to the research lab and data from the study due to the worldwide COVID-19 pandemic, data analysis for the study was limited. Specifically, analysis of the video-recorded behavioral data for the elevated plus maze and open field test was unable to be conducted. Although the behavioral data was inaccessible, the weight differences observed between groups were identified as an important factor to consider when attempting to analyze behavior.

Behavioral Data

Behavioral Observation (Personal Observations). While observing behavior of the rats during the experimental protocol at times of feedings and handling, there appeared to be behavioral differences that would have been identified had analysis of the video behavioral data from the elevated plus maze and open field test been conducted. While these types of observations could have been impacted by researcher bias or other confounds, it appeared that tamoxifen rats exhibited greater anxiety in response to handling by researchers that seemed to be almost an aversion to being handled at all by the researchers. This reactivity was not limited to a
single investigator but instead was noted by all investigators as tamoxifen rats exhibited an aversion to handling or being held at all, generally refusing to rest in the crook of the researchers’ arms.

Weight Data

The two different treatment regimens in the study were associated with significantly different effects on the weight of the rats. In summary, tamoxifen treatment was associated with significant weight loss whereas normal feeding in the control group was associated with weight maintenance and consistently increasing weight. Two rats receiving tamoxifen treatment in both Cohort 1 and Cohort 2 were removed from the experimental protocol as a result of health concerns related to unmitigated weight loss. Following removal from the study, tamoxifen treatment in these rats was ended and their medicated tamoxifen pellets were replaced by normal unmedicated rat chow. Their weight was monitored following removal to ensure weight loss was mitigated and a gradual weight gain was noticed following reinstatement of feeding with normal rat chow.

Cohort 1

In Cohort 1, the mean starting weight for tamoxifen rats was 254 g while the mean starting weight for control rats was 260.25 g. Following the 13-week experimental protocol, the mean weight for tamoxifen rats was 270.67 g while the mean weight for control rats was 346.13 g. In Cohort 1, initiation of tamoxifen treatment was associated with significant weight loss in the rats which was eventually stabilized and mitigated, most likely corresponding to feeding using food mash with diet gel (Figure 1). The weight of the tamoxifen rats stabilized following the initial weight loss and increased during the last few weeks of treatment. Control rats in Cohort 1 exhibited steady weight gains during the experimental protocol as they were fed with
unmedicated normal rat chow (Figure 1). An independent samples \( t \)-test determined that there was a significant difference in weight change between starting weight and weight at behavioral testing, \( t(12) = 2.18, p < .0001 \) between tamoxifen rats (\( M=16.67, SD=3.25 \)) and control rats (\( M=85.88, SD=17.59 \)) in Cohort 1.

Figure 1. Cohort 1 mean tamoxifen and control rat weight change between starting weight measured before beginning of experimental protocol and ending weight measured 24 hours before behavioral testing.

Cohort 2

In Cohort 2, the basal starting weights for the rats were higher because these rats were older at the start of the experimental protocol. Tamoxifen rats in Cohort 2 had a mean starting weight of 312.20 g and control rats in Cohort 2 had a mean starting weight of 313.38 g. Following conclusion of the experimental protocol, tamoxifen rats had a mean weight of 282.30 g while control rats had a mean weight of 383.88 g. In Cohort 2, tamoxifen treatment was associated with significant weight loss following initiation of treatment that may have been associated with a greater starting basal weight of the rats because of their older age. Eventually, weight loss was mitigated, and the weights of the tamoxifen rats stabilized (Figure 2). Control
rats in Cohort 2 exhibited steady weight gain over the course of the experimental protocol as a result of their diet consisting of unmedicated normal rat chow (Figure 2). An independent samples $t$-test determined that there was a significant difference in weight change between starting weight and weight at behavioral testing, $t(16)=2.12, p<.0001$, between tamoxifen rats ($M=-29.90, SD=15.27$) and control rats ($M=70.50, SD=32.45$) in Cohort 2.

![Figure 2. Cohort 2 mean tamoxifen and control rat weight change between starting weight measured before beginning of experimental protocol and ending weight measured 24 hours before behavioral testing.](image)

Comparison between the weight changes of tamoxifen rats in the two cohorts is represented in Figure 3. The mean starting weight of tamoxifen rats in Cohort 1 of 254 g was significantly different from the mean starting weight of tamoxifen rats in Cohort 2 of 312.2 g ($t(14)=2.14, p<.0001$). Although the mean starting weights of tamoxifen rats in the two cohorts were significantly different, the mean ending weights of the tamoxifen rats in the two cohorts were relatively similar being 270.67 g and 282.30 g respectively (Figure 3). The mean
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differences in weight change for the two treatment conditions in the two cohorts are represented in Figure 4. It can be seen that tamoxifen rats in Cohort 1 that had a significantly lower starting weight than rats in Cohort 2, ended up gaining weight on average over the course of the experimental protocol whereas the rats in Cohort 2 lost weight on average (Figure 4). With the increased weight loss observed in Cohort 2 due an increased basal starting weight of the tamoxifen rats, it was important that the BCS scale was implemented as a health monitoring measure in the experimental protocol. Many of the tamoxifen rats in Cohort 2 would have been required to be removed from the study because of the weight loss percentage relative to their starting body weight at the beginning of the protocol. Using the BCS scale ensured the safe monitoring of the health of the rats in the study as it was a much more reliable and less variable measure than the weight of the rats in the study.

Figure 3. Mean tamoxifen rat weight change in the two cohorts between starting weight measured before beginning of experimental protocol and ending weight measured 24 hours before behavioral testing.
Discussion

The purpose of this study was to determine whether long-term administration of tamoxifen over the course of 12 weeks in female rats was associated with behavioral effects compared to rats who did not undergo tamoxifen treatment. It was hypothesized that behavior of female rats would be negatively affected by long-term tamoxifen treatment based on the antagonistic effects of tamoxifen on estrogen receptors and the role estrogen plays in the brain, as well as its association with behavior. As a result of the effects of tamoxifen, it was expected that these effects on behavior would be manifested in increased anxiety and depressive behaviors observed using the elevated plus maze and open field test behavioral paradigms. In the elevated plus maze, increased anxiety behavior that was expected to be observed as a result of tamoxifen treatment included greater time spent in the enclosed arms of the maze and less time spent in the open arms and a greater number of entries into the enclosed arms with a lower number of entries into the open arms of the maze. In the open field test, increased anxiety and depressive behavior that was expected to be associated with long-term tamoxifen treatment included a lower number
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of central crossings in the open field, greater time spent on the outer parts of the open field near the walls and corners, and a lower amount of movement around the open field (Pellow et al., 1985; Seibenhener & Wooten, 2015; Valvassori et al., 2017; Walf & Frye, 2007).

Due to the COVID-19 pandemic our experiment was shortened in the Cohort 2 experimental protocol, and as a result the video-recorded behavioral data from the elevated plus maze and open field test was unavailable for formal analysis using the Noldus Etho-Vision-XT system (Noldus Information Technology, the Netherlands, v.14). Based on observation of the rats during the experimental protocol, it appeared that tamoxifen rats exhibited an aversion to handling that could have been indicative of increased anxiety. This was expected to be manifested in the behavioral data obtained from the elevated plus maze and open field test. While this observational experience with the rats includes possible researcher bias and in no way identifies any association between tamoxifen treatment and increased anxiety behavior, it emphasizes the need to analyze the video data from the behavioral assessments in the future to achieve an unbiased and structural comparison between the two treatment conditions and their effects on behavior.

Assessing Behavior

The elevated plus maze and open field test were used to assess the behavioral effects of long-term tamoxifen treatment in this study. Anxiety is assessed in the elevated plus maze by comparing the number of entries and the amount of time spent in the open arms of the maze relative to the enclosed arms with increased anxiety associated with more time spent in the enclosed arms of the maze (Bailey & Crawley, 2009; Pellow et al., 1985). Anxiety and depressive behaviors are assessed using the open field test by analyzing locomotor and exploratory behaviors. Generally, increased time spent in the periphery of the open field and a
decreased number of central crossings are associated with increased levels of anxiety (Seibenhener & Wooten, 2015). Both test paradigms are based on the tendency for rats to explore a novel environment while also having an aversion to potentially threatening and dangerous environments, like brightly lit and open spaces as in the open arms of the maze and the center of the open field where rats could be exposed to predators. While both the elevated plus maze and open field test are used to assess anxiety, there are many different behavioral components that must be considered when analyzing behavioral data from these tests. It is important to assess locomotor activity for the different groups of rats while also classifying and identifying the specific indices of anxiety during the behavioral tests, including exploratory behaviors and movement throughout the different apparatuses. These assessments of behavior should be the focus of future behavioral data analysis both from the video obtained prior to the termination of this study’s protocol as well as in similarly designed studies conducted in the future.

**Observed Weight Differences**

The trends in weight data were analyzed for the two treatment conditions over the two cohorts in the study. In both cohorts the control rats that received unmedicated rat chow for the duration of the study showed a steady increase in their weights over the 10-13 weeks. The tamoxifen rats in both cohorts exhibited an initial decline in body weight for about 2 weeks that corresponded to the initiation of tamoxifen treatment. Following the initial decrease in body weight, the tamoxifen rats then regained weight and eventually had their body weights relatively stabilize toward the end of the 10-13-week protocol. Interestingly, although there was a large difference between the mean starting weights for tamoxifen rats in Cohort 1 and tamoxifen rats in Cohort 2, 254 g versus 312.2 g respectively, tamoxifen rats in both cohorts ended with
relatively similar end weights following tamoxifen treatment, 270.67 g and 282.30 g respectively. This supports the effects of tamoxifen treatment on metabolism that significantly alter weight, as observed in the female rats in the study, as well as the importance of the use of the BCS scale in monitoring the health of the rats in Cohort 2 of this study. If weight had been the sole factor used in health monitoring in Cohort 2, many of the tamoxifen rats would have been removed from the study as a result of the increased percentage of body weight lost relative to their initial weight at the beginning of the experimental protocol. The BCS scale ensured the safety of the rats in the study as well as prevented the loss of tamoxifen rats because of increased weight loss.

In a previous study, rats injected with tamoxifen had a similar reduction in body weight that corresponded to decreased standard chow intake and a decrease in retroperitoneal fat (Lampert et al., 2013). Interestingly, this study also found that rats treated with tamoxifen decreased in body weight even when they had the same caloric intake as control rats, providing evidence of low caloric efficiency and supporting tamoxifen’s effects on metabolism (Lampert et al., 2013). While caloric intake was not tracked in this study, tamoxifen rats and control rats had the same access to food throughout the study so a significant difference in caloric intake between the two treatments would not be expected. Through scoring on the BCS scale as well as observation during perfusion of the rats, it was evident that control rats had significantly more retroperitoneal fat than tamoxifen rats.

Implications for Behavioral Analysis. The analysis of weight data from this study have interesting implications for future study and the results of this study. Tamoxifen treatment as evidenced in this study and the past literature causes weight reduction resulting from changes in metabolism (Lampert et al., 2013). It was assumed that the caloric intake of rats in both
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treatment conditions was relatively similar during the study, but it may be a factor to consider tracking in future studies that administer tamoxifen through medicated food pellets. Additionally, weight changes in rats resulting from tamoxifen treatment and standard rat chow may introduce a confound to behavior based on the weight changes associated with each treatment. This is an interesting factor that is important to consider when studying differences in behavior, however it is expected that differences in weight did not confound the treatment effects of this study because both treatment conditions were provided with the same amounts of food and were expected to have relatively similar levels of caloric intake. Weight gain is a factor that may affect studies lasting longer than 10-weeks since weight gain observed in control rats will continue as rats age, making the possibility of confounding behavioral effects caused by weight more likely.

A previous study found that metabolic syndrome (MS) in rats associated with significantly increased body weight arising from a high-sucrose diet led to associated anxiety-like behaviors observed in the open field test (Rebolledo-Solleiro et al., 2017). The rats studied, however, were male while the rats in the present study were female. Additionally, in the study conducted by Rebolledo-Solleiro et al. (2017), both the experimental and control rats were fed with standard rat chow as was done in the experimental protocol of the present study. Given that sucrose-infused drinking water, in addition to standard rat chow, was needed to induce MS in these middle-aged rats, it is unlikely that the weight increase in the control rats in this study was significant enough to induce MS and possibly induce confounding behavioral effects. Weight should be a factor considered in future studies examining anxiety-like behavior because significant increases in weight and obesity have been associated with anxiety-like behaviors in female rats (da Costa Estrela et al., 2015). In future studies analyzing the effects of tamoxifen relative to control rats, measures can be taken to mitigate the possible effects of weight and
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obesity on behavior, including a younger control group with lower starting basal weights or the use of enriched environments for both groups of rats to mitigate expected weight gain in control rats.

**Future Directions: Our Path Forward after Covid-19**

An important factor in the validity of this study was the amount of tamoxifen onboard the rats. It is important to establish a significant difference in the levels of tamoxifen between the two treatment conditions at the conclusion of the study to ensure tamoxifen treatment could be associated with any observed behavioral effects. In this study, blood draws were conducted prior to perfusion to analyze blood samples for tamoxifen metabolites. Because of the COVID-19 pandemic, blood samples were unable to be analyzed. The analysis of tamoxifen metabolites in blood samples, however, is an important factor for future study. Future analysis of the data in this study will also include analysis of tamoxifen metabolite data using the rapid LC-ESI-MS/MS method in collaboration with a metabolic laboratory at the Children’s Hospital of Philadelphia (Rama Raju et al., 2015).

In addition to the behavioral effects of tamoxifen, cognitive effects of long-term tamoxifen treatment are an important area for future study. Breast cancer patients treated with tamoxifen have presented more often clinically with memory problems than patients not treated with tamoxifen (Bender et al., 2006; Jenkins et al., 2004; Paganini-Hill & Clark, 2000). Studies using neuropsychological tests to assess the effects of tamoxifen on cognition found that breast cancer patients treated with tamoxifen performed significantly worse than healthy controls on measures of verbal memory, executive functioning, and semantic memory as well as having a nearly significant decrease in hippocampal volume measured using brain imaging studies of subjects (Jamie L. Eberling et al., 2004; Schilder et al., 2010). These types of cognitive effects of
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tamoxifen treatment were supported by studies in animal models. Tamoxifen treatment has been found to impair memory consolidation and memory retrieval processes in female rats (Chen et al., 2002; Lichtenfels et al., 2017). The cognitive effects associated with tamoxifen treatment in humans and animal models are hypothesized to be associated with tamoxifen’s effect on estrogen activity in the brain because of the neuroprotective effects of estrogen. Treatment with estrogen has been associated with improved cognitive scores in female dementia patients and estrogen replacement therapy following menopause has been associated with increased hippocampal volumes (J. L. Eberling et al., 2000; Ohkura et al., 1994). This is interesting given that tamoxifen was found to be associated with a trend of decreasing hippocampal volumes in an imaging study of human patients (Jamie L. Eberling et al., 2004). The neuroprotective effects of estrogen have also been observed in rodent models with estrogen treatments associated with improved performance in cognitive memory tasks following ovariectomy, removal of the estrogen-producing ovaries, which has been associated with memory deficits and impairments (Djiogue et al., 2018; Fan et al., 2010; Fernandez et al., 2008; Fortress et al., 2013).

Based on the past literature establishing the cognitive effects associated with tamoxifen treatment both in human patients and animal models, as well as the noted effects of tamoxifen on estrogen and the important neuroprotective effects of estrogen in both humans and animal models, a critical future area of study is the investigation of the cognitive effects of long-term tamoxifen treatment. An effective model for the future study of long-term tamoxifen treatment is female rats. Cognitive measures of short-term memory, long-term memory, and social memory and behavior can be assessed using the novel object recognition task and social recognition task as cognitive assessment paradigms. Additionally, following future study of the cognitive and behavioral effects of tamoxifen treatment in female rats, assessment of the brains using
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immunocytochemistry would provide insight to the underlying neurobiological effects of tamoxifen treatment that may elicit some of the cognitive and behavioral effects associated with the drug. Based on the literature it is believed that cognitive and behavioral effects of tamoxifen treatment are mediated by corresponding hippocampal circuitry. Anxiety cells have been identified in the ventral CA1 region of the hippocampus with neural networks between the ventral hippocampus and lateral hypothalamic area associated with the manifestation of anxiety behaviors in rodents (Jimenez et al., 2018). vCA1 neurons were found to be activated during anxiety-associated behaviors, specifically the exploration of the open arms of the elevated plus maze relative to the enclosed arms (Jimenez et al., 2018). An effective marker for assessing hippocampal circuitry relevant to cognition and behavior would be Tuj-1. Tuj-1, a marker of immature neurons, would be an effective stain to analyze the process of neurogenesis, the creation and development of new neurons throughout the lifespan, in the hippocampi of female rats following long-term tamoxifen treatment (von Bohlen und Halbach, 2007).

Conclusion

Tamoxifen is a widely prescribed treatment for hormone-receptor positive breast cancer with a multidimensional mechanism of action and extended treatment window sometimes lasting longer than a decade in patients. Even with the significant impact tamoxifen has on estrogen activity and the important neuroprotective effects of estrogen on the brain, the behavioral and cognitive effects of tamoxifen have been relatively unexplored in research. This study confirmed the effectiveness of administering tamoxifen through medicated food pellets to female rats in order to study the effects of long-term tamoxifen treatment. 10-13-week tamoxifen treatment was associated with significant differences in weight change in tamoxifen rats relative to control group rats who received unmedicated rat chow for the duration of the study. Unfortunately, due
towards the COVID-19 pandemic, the behavioral data recorded of the rats completing the elevated plus maze and open field test was unable to be analyzed. Future research should further this study by assessing the behavioral data obtained in this study as well as designing similar experiments to analyze the cognitive effects of long-term tamoxifen treatment. Future studies can utilize this study as a proof-of-concept of the administration of tamoxifen through medicated food pellets over an extended treatment period lasting over 10 weeks. The results of this study, however, indicate that future investigations should consider the significant weight change that results from long-term tamoxifen treatment relative to control rats fed with standard rat chow, as weight differences could confound effects on behavior from the treatment. Additionally, similar studies should confirm the levels of tamoxifen metabolites onboard treated rats to ensure a clinically relevant dosage as was planned in this study’s protocol prior to its interruption due to COVID-19. The future study of underlying hippocampal structures is also important because hippocampal circuitry may provide insight regarding the effects of long-term tamoxifen treatment on behavior and cognition. Future studies like this one will help continue to build understanding of the breast cancer drug tamoxifen and the possible effects it may have on the behavior and cognition of patients treated with the drug.

Tamoxifen is an important treatment for hormone-receptor positive breast cancer that is prescribed to many women for an extended treatment window that can extend longer than 10 years. Patients receiving tamoxifen treatment have reported behavioral and cognitive effects of the drug to physicians, while studies in animal models have also identified behavioral and cognitive impairments caused by tamoxifen. There still remains, however, relatively few studies on the effects of long-term tamoxifen treatment that effectively model a clinically relevant treatment window that is used in patients being treated for hormone-receptor positive breast
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Cancer. The mechanism of action of tamoxifen as a selective estrogen receptor modulator has the potential to affect behavior and cognition through the drug’s effects on estrogen, a vital neuroprotective hormone for the brain and cognitive health of women throughout life. Based on the widespread use of tamoxifen as an adjuvant hormone therapy for the treatment of breast cancer and the importance of estrogen in the cognitive health of women, this study sought to investigate the effects of long-term tamoxifen treatment on behavior in Long-Evans Hooded female rats. Similar studies in the future should build upon the present study to further investigate the effects of long-term tamoxifen on behavior and cognition as developments in this area of research will have important implications for the cognitive health of many women who use tamoxifen as an adjuvant therapy for the treatment of breast cancer.
References


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Appendix A

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<tr>
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<th>Tamoxifen Treatment</th>
<th>Unmedicated Food Treatment</th>
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<tbody>
<tr>
<td>Cohort 1</td>
<td>8*</td>
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<tr>
<td>Cohort 2</td>
<td>12</td>
<td>8</td>
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Table 1. Overview of rats assigned to the two treatment groups for Cohort 1 and Cohort 2.

Figure 1. Cohort 1 mean tamoxifen and control rat weight change between starting weight measured before beginning of experimental protocol and ending weight measured 24 hours before behavioral testing.
Figure 2. Cohort 2 mean tamoxifen and control rat weight change between starting weight measured before beginning of experimental protocol and ending weight measured 24 hours before behavioral testing.
Figure 3. Mean tamoxifen rat weight change in the two cohorts between starting weight measured before beginning of experimental protocol and ending weight measured 24 hours before behavioral testing.

Figure 4. Mean weight changes for tamoxifen and control rats in Cohort 1 and Cohort 2.
Appendix B: Representative Body Condition Scoring (BCS) charts for rodents

**MICE**

**BC 1**
*Mouse is emaciated*
- Skeletal structure extremely prominent, little or no flesh cover
- Vertebrae distinctly segmented

**BC 2**
*Mouse is under-conditioned*
- Segmentation of vertebral column evident
- Dorsal pelvic bones are readily palpable

**BC 3**
*Mouse is well-conditioned*
- Vertebrae and dorsal pelvis not prominent, palpable with slight pressure

**BC 4**
*Mouse is over-conditioned*
- Spine is a continuous column
- Vertebrae palpable only with firm pressure

**RATS**

**BC 1**
*Rat is emaciated*
- Segmentation of vertebral column prominent if not visible
- Little or no flesh cover over dorsal pelvis, pins prominent if not visible
- Segmentation of caudal vertebrae prominent

**BC 2**
*Rat is under-conditioned*
- Segmentation of vertebral column prominent
- Thin flesh cover over dorsal pelvis, little subcutaneous fat, pins easily palpable
- Thin flesh cover over caudal vertebrae, segmentation palpable with slight pressure

**BC 3**
*Rat is well-conditioned*
- Segmentation of vertebral column easily palpable
- Moderate subcutaneous fat store over pelvis, pins easily palpable with slight pressure
- Moderate fat store around tail base, caudal vertebrae may be palpable but not segmented

**BC 4**
*Rat is over-conditioned*
- Segmentation of vertebral column palpable with slight pressure
- Thick subcutaneous fat store over dorsal pelvis, pins of pelvis palpable with firm pressure
- Thick fat store over tail base, caudal vertebrae not palpable
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**Mouse is obese**
- Bone structure disappears under flesh and subcutaneous fat

**Rat is obese**
- Segmentation of vertebral column palpable with firm pressure, may be a continuous column
- Thick subcutaneous fat store over dorsal pelvis, pins of pelvis not palpable with firm pressure
- Thick fat store over tail base, caudal vertebrae not palpable


Adapted from: Hickman D, Swan M. 2010. Use of a Body Condition Score Technique to Assess Health Status in a Rat Model of Polycystic Kidney Disease, *JAALAS 49(2)* 155-159

*Note: BCS should be extrapolated to the particular species approved in your IACUC protocol*