ABSTRACT

The Effects of Chemotherapy on Affect and Behavior in Mice

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Chemotherapy has been shown to disrupt cognition in breast cancer patients, referred to as chemotherapy-induced cognitive impairment (CICI). CICI may be caused by inflammatory cytokines or decreased hippocampal neurogenesis as a result of chemotherapy toxicity, but exercise has been shown to be successful in reducing these effects. CICI is also accompanied by affective disruption, but has not been as extensively studied. This study uses behavioral tests to examine the effects of chemotherapy and exercise on anxiety-like and depressive-like behaviors in mice. The results indicate that chemotherapy causes affective disturbance in mice, meaning that it may be causing anxiety and depression in breast cancer patients. The results also show that exercise may be preventative against breast cancer patients developing anxiety and depression.

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THE EFFECTS OF CHEMOTHERAPY ON AFFECT AND BEHAVIOR IN MICE

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For my parents. Thank you for making my education at Baylor University possible. Everything I have accomplished has been because of your unconditional love and support.

LIST OF ABBREVIATIONS

BBB	Blood Brain Barrier
CAL	Conditioned Associative Learning
Chemo	Chemotherapy
CICI	Chemotherapy-Induced Cognitive Impairment
EPM	Elevated Plus Maze
FST	Forced Swim Test
NMTS	Non-matching-to-sample
OF	Open Field
SA	Social Approach
Sal	Saline
Sed	Sedentary
Tg	Transgenic
WT	Wild Type

CHAPTER ONE

Literature Review

Cancer and Chemotherapy

According to the American Cancer Society, the incidence of breast cancer has been slowly increasing since 2004. There are many risk factors correlated with this increase, including alcohol consumption, age, less childbearing, and social class (Key et al., 2001). Among the ages of 40-50, it may simply be because more people are being screened for it (McPherson et al., 2000). It seems, however, that breast cancer may not be disrupting lives on its own, because a method of treating breast cancer, chemotherapy, has also been affecting the lives of cancer patients. Even though chemotherapy treatment creates a better prognosis for breast cancer, it is also associated with toxicity and can cause lasting effects on their quality of life.

There are four molecular subtypes of breast cancer and each responds differently to treatment (Masood, 2016). Treatment is decided based on tumor stage, tumor grade, hormone receptor status, and HER-2 receptor status. There are two types of estrogen receptor positive tumors, luminal A and luminal B. Luminal A tumors are HER-2 receptor negative and account for up to 45% of breast cancers (Voduc et al., 2010). Luminal A tumors have the best prognosis and low recurrence rates. Luminal B tumors may be HER-2 receptor positive or negative and are usually diagnosed at a younger age than luminal A tumors (Metzger et al., 2010). Luminal B tumors account for up to 20% of breast cancers and tend to have high survival rates, but not as high as luminal A tumors. Luminal A and B tumors respond well to endocrine therapy (Masood, 2016). The

next subtype is basal-like/triple negative tumors which have little to no expression of HER-2/neu oncogene and estrogen and progesterone receptors, but they have high expression of basal epithelial cell layer gene characteristics and expression of cytokeratin 5, 6, 17 and integrin-B4 (Masood, 2016). About 20% of breast cancer tumors are triple-negative/basal-like and most BRCA-1 gene mutation related breast cancers are triple-negative/basal-like (Atchley et al., 2008). The prognosis for this subtype is not as high as it is for luminal A and B tumors. It is usually treated with a combination of surgery, radiation, and chemotherapy. The HER-2/neu oncogene positive tumors express HER-2/neu oncogene receptors, but they are estrogen and progesterone receptor negative. This subtype accounts for up to 15% of breast cancers (Voduc et al., 2010). HER-2 enriched breast cancers are treated with chemotherapy and trastuzumab, a HER-2 targeted therapy (Gianni et al., 2011).

One of the more studied effects caused by chemotherapy is "chemobrain." Many breast cancer patients report cognitive deficits after receiving chemotherapy treatment. These cognitive impairments, referred to as "chemobrain" or chemotherapy-induced cognitive impairments (CICI), include dysfunction in memory, executive functioning, learning, and concentration (Argyriou et al., 2011; Moore, 2014). CICI has been reported to persist for up to ten years after cessation of treatment (Ahles & Saykin, 2002; Ruiter et al., 2011). CICI is associated with long-term high doses of chemotherapy treatment with doxorubicin, paclitaxel, cyclophosphamide, and fluorouracil (Ahles & Saykin, 2002; Cheng et al., 2013).

However, not every breast cancer patient who undergoes chemotherapy treatment develops CICI. It is estimated that 20% to 30% of breast cancer survivors experience

cognitive dysfunction (Huang et al., 2019). Numerous biomarkers have been associated with increased risk of CICI, such as the e4 allele of the apolipoprotein E (APOEe4) (Ahles & Saykin, 2002). Cytokines involved with inflammation in the immune system, such as IL-6, may also play a role (Argyriou et al., 2011, Lee et al., 2004). One hypothesis is that dysregulation of the immune system caused by the cancer or chemotherapy may cause an increase of these inflammatory cytokines that cross the blood-brain barrier (BBB) (Argyriou et al., 2011). This increase is directly correlated with cognitive deficits in the areas of executive function and spatial ability.

Affective Symptoms of CICI in Humans

A study on breast cancer survivors one year after treatment revealed that 9.4 - 66.4% experienced depressive symptoms and 17.9 - 33.3% experienced anxiety symptoms (Maass et al., 2015). These symptoms may arise from the stress of the illness, but they continue to present for up to five years after treatment in a subset of patients (Lee et al., 2004). People with depression and anxiety exhibit higher levels of inflammatory cytokines, similar to the increase induced by chemotherapy treatment (Charlton, 2000; Pyter al., 2017). Poor self-appraisal associated with negative affect contributes to the perception of decreased cognitive ability (Hermelink et al., 2010).

Recent research has found a strong correlation between depression and self-reported CICI. However, the relationship between depression and cognitive impairments is not well-established (Huang et al., 2019; Pinto & de Azambuja, 2011; Hermelink et al., 2010). In fact, those affected usually are unaware of their cognitive impairments (Hermelink et al., 2010). Prevalence of depression among breast cancer patients increases

after diagnosis and during treatment, but is hard to determine because of differences in definition and evaluation methods between studies (Pinto & de Azambuja, 2011). One study found 37.5% of breast cancer patients to have clinical depression, but it could be as high as 46% (Massie, 2004; Reece et al., 2013). Furthermore, depression has been shown to last beyond chemotherapy treatment. Women who have undergone chemotherapy treatment for breast cancer have a higher prevalence of depression and are at an increased risk of depression compared to the general female population (Maass et al., 2015; Suppli et al., 2014). The quality of life of those who report CICI, usually the ones suffering from negative affect, is more affected than the quality of life of those who have CICI but are unaware of it (Moore, 2014). Furthermore, while most data indicates that anxiety levels decline after the start of chemotherapy treatment, anxiety has been correlated with higher levels of depression (Reece et al., 2013, Maass et al., 2015). While anxiety and depression in cancer patients have been studied at great lengths, the cause has mostly been attributed to a combination of the toll of illness, stress, and cytotoxic treatment (Pyter et al., 2017). A prospective study has shown a relationship between cytotoxic treatment and depression in breast cancer patients, but a causal factor has yet to be identified (Cvetkovic & Nenadovic, 2016).

Animal Models of CICI

Animal models have been used to study the cognitive deficits associated with chemotherapy. A transgenic model of breast cancer has been created in mice that produces tumorigenesis similar to that in humans (Guy et al., 1992). This model has been used to study the effects chemotherapy treatments such as doxorubicin and

cyclophosphamide have on cognition. Doxorubicin is not able to cross the BBB in significant amounts, so its ability to cause such intense effects shows the potency of the toxicity. Genetic variations in BBB transporters, specifically those associated with weaker DNA-repair mechanisms, allow small amounts to enter the brain parenchyma (Argyriou et al., 2011). Even with such small amounts, it was shown that chemotherapy can produce cognitive deficits independent of illness in the areas of spatial memory, non-matching-to-sample (NMTS), and conditional associative learning (CAL) tasks (Winocur et al., 2018; Kitamura et al., 2015).

There has also been mixed evidence of anxiety and depressive-like symptoms in breast cancer animal models (Kitamura et al., 2015; Pyter et al., 2017). Kitamura and colleagues (2015) found that rats treated with doxorubicin and cyclophosphamide displayed significantly more anxiety-like behaviors compared to those treated with saline. In a model of breast cancer survivorship in mice that utilizes the removal of a non-metastatic tumor, behavioral changes persisted beyond tumor reduction as measured by the elevated-plus maze and marble-burying tests (Pyter et al., 2017). The inflammatory cytokine levels of the rodents in this study remained high after the tumor resections, suggesting that changes to the immune system may be involved in the lasting effects on behavior. Doxorubicin and cyclophosphamide have been shown to not only cause cognitive impairment, but to induce anxiety-like behavior in rats as well (Kitamura et al., 2015). But in another study, while chemotherapy drug thioTEPA created deficits in learning and memory and induced stress, there was no significant difference in depressive-like behaviors between the chemotherapy treated group and the saline treated group (Mondie et al., 2010). Another study using paclitaxel, however, did show

chemotherapy-induced depressive-like behaviors alongside cognitive impairment, but using novel object recognition and sucrose preference tests as measures (Walker et al., 2017). While the results are still unclear, these data suggest the burden of illness and treatment may have long-lasting effects on quality of life.

The affective symptoms during and after chemotherapy are important to study because they may be due more to the treatment of cancer than the illness itself. Given the cognitive deficits chemotherapy has on cancer patients, chemotherapy may independently be a causal factor in affective disorders. To improve the quality of life of breast cancer patients, the cause of these symptoms should be studied so a proper course of treatment or preventative measures can be determined.

Exercise as an Intervention Against the Development of CICI

Evidence suggests that exercise is a possible preventative measure that can be taken against the development of CICI. Exercise has many health benefits for breast cancer patients. Firstly, it is reduces risk against developing breast cancer (Veljkovic et al., 2011). It is estimated that a few hours of exercise each week decreases risk of breast cancer by 30% (Key et al., 2001). A study conducted on cardiotoxicity in breast cancer patients showed that women suffering from chemotherapy-induced cardiac dysfunction benefit from exercise (Bonsignore et al., 2014). Secondly, exercise has also been shown to increase quality of life during and following chemotherapy treatment (Pinto & de Azambuja, 2011). Exercise can increase physical self-perceptions and quality of life in breast cancer survivors, showing that it may be influential in treating negative affect (Perkins et al., 2011).

A proposed intervention for CICI is exercise because of its success in treating other symptoms. Not only does exercise have preventative effects for breast cancer by promoting the immune system to create antibodies, but it has also been shown to alleviate the symptoms of CICI (Veljkovic et al., 2011). Winocur and colleagues (2014) showed that rats performed better on spatial memory and NMTS tasks after exercise and that exercise was successful in preventing and treating CICI. Chemotherapy treatment suppresses hippocampal neurogenesis, and the effectiveness of exercise is partly due to the prevention of that. A mechanism of antidepressants is to increase hippocampal neurogenesis, and a study using a mouse model of stress demonstrated that increasing hippocampal neurogenesis is sufficient to decrease anxiety and depressive-like behaviors (Hill et al., 2015). However, more research still needs to be done on the effectiveness of treating affective symptoms of CICI with exercis

CHAPTER TWO

Hypothesis

Since previous research has produced mixed results on whether chemotherapy independently causes affective impairments in mice, this study examines the anxiety-like and depressive-like behaviors in mice treated with chemotherapy. Specifically, this study examines the effects of doxorubicin and cyclophosphamide. A mouse model of breast cancer will be used alongside a wild type mouse to compare the interaction between illness and chemotherapy treatment on behavior. This study will also examine the effects of exercise on the affective symptoms of CICI. The purpose of this study is to examine whether chemotherapy treatments alone cause affective disruption in the mouse model of breast cancer, and if exercise is an effective treatment for alleviating symptoms.

It is hypothesized that doxorubicin and cyclophosphamide play a causal role in the depressive-like and anxiety-like symptoms in both the transgenic and wild type mice. Given the success of exercise stimulating hippocampal neurogenesis, it is also hypothesized that exercise in the form of voluntary wheel running will be effective in alleviating depressive-like and anxiety-like behaviors.

CHAPTER THREE

Methods

Subjects

All subjects used were female FVB/N-Tg (MMTV-neu) 202Mul/J (transgenic, Tg), or FVB/N (wild-type, WT) mice from Jackson Laboratory in Bar Harbour, Maine (Guy et al., 1992). FVB/N-Tg mice develop breast tumors within 6-9 months of age. MMTV-neu mice overexpress the wild type rodent neu oncogene, which is equivalent to HER-2/neu oncogene positive tumors (Winocur et al., 2018). Mice were housed with standard bedding and *ad libitum* access to food and water. The mice were assigned to different rearing conditions: exercise or sedentary. In the exercise group, the mice were housed with a running wheel, while in the sedentary control group, mice were housed with a locked, nonfunctional wheel.

The subjects were randomly assigned to chemotherapy or saline treatment groups (*Figure 1*). The mice in the chemotherapy groups were given three weekly injections of 40 mg/kg cyclophosphamide and 4 mg/kg doxorubicin intraperitoneally. The mice in the saline control group were injected with equal amounts of saline.

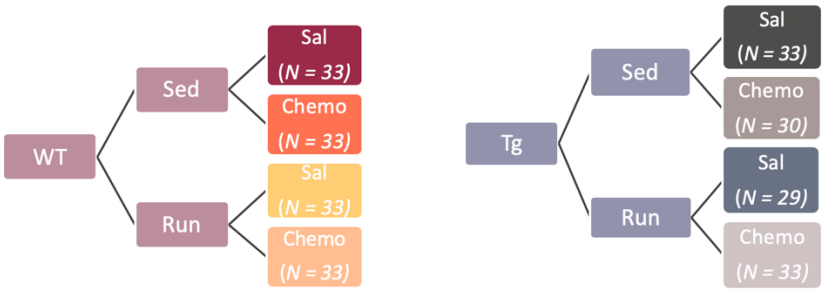

Figure 1. Schematic of experimental groups. 2 (WT vs Tg) x 2 (Sed vs Run) x 2 (Saline vs Chemo) factorial design was used, n = 29-33 mice/group. Figure courtesy of Kayla Gilley.

Behavioral Tasks

The mice underwent a variety of behavioral testing to assess anxiety and depressive-like behavior. These tasks included the 3-Chambered Social Approach, Elevated Plus Maze, and Open Field for anxiety-like behavior and Forced Swim Test for depressive-like behavior. Baseline behavioral testing was performed after 6 months and second battery or post-treatment behavioral testing was conducted one month following the completion of treatment (*Figure 2*).

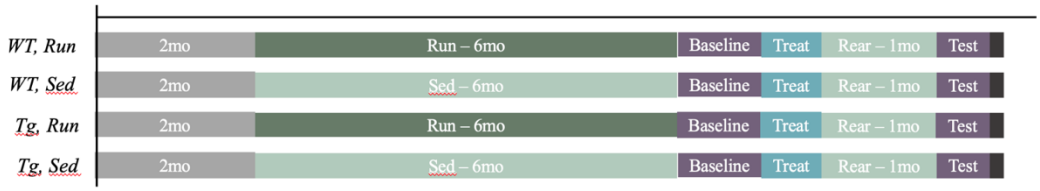

Figure 2. Schematic of experimental timeline. Figure courtesy of Kayla Gilley. Mice were assigned to a rearing condition (Run or Sed) for 6 months, followed by baseline behavioral testing, treatment (Chemo or Sal), one month of sedentary rearing, and post-treatment behavioral testing.

In the 3-Chambered Social Approach task (SA), anxiety-like behavior can be determined through the amount of social interaction displayed by the mouse (Toth & Neumann, 2013). The arena has three chambers (17” width x 8” length x 9” height) connected by a sliding door on both sides of the center chamber. In each of the side chambers there is an isolation cage. One isolation cage contained a mouse while the other remained empty. The mouse started the test in the center chamber with the sliding doors closed and was given five minutes to habituate to the chamber. In the next five minutes, the chamber doors were opened and the mouse was free to explore all three chambers.

The amount of time the mouse spent in each chamber was analyzed on video using the SMART video tracking software. The amount of time the mouse spent in the empty chamber was compared to the time the mouse spent in the chamber containing the other mouse. Less time spent in the chamber with the other mouse indicates social avoidance, a measure of anxiety-like behavior in mice (Toth & Neumann, 2013).

The next measure of anxiety-like behavior in the mice is the Elevated Plus Maze (EPM) (Hogg, 1996). This task apparatus has two open arms measured 12" long x 2" wide x 0" high with no side barriers and two closed arms 12" long x 2" wide x 6" high that have side barriers that extend from a central platform of 2" x 2". The apparatus is elevated 50 centimeters above the floor. The mouse was placed on the central platform facing an open arm and given five minutes to explore the maze. SMART video tracking was used to calculate the number of times the mouse entered each arm and the amount of time the mouse spent in each arm. More time spent in closed arms is indicative of anxiety-like behavior (Landauer & Balster, 1982).

In the Open Field task (OF), mice were placed in an open arena of 17.7" x 17.7" x 15.7" for 15 minutes. SMART video tracking was used to analyze the activity of the mouse in the arena, measuring distance travelled and amount of time spent in different areas of the open field. Less exploring is indicative of anxiety-like behavior (Prut et al., 2003).

The Forced Swim Test (FST) is a task frequently used to measure depressive-like behavior through behavioral despair (Porsolt et al., 1977, 1978). The mouse was placed in a chamber for six minutes that was filled halfway with room temperature water. The chamber is 4" in diameter and 10" high. Activity was measured using SMART video

tracking software. The amount of immobility was calculated and used to calculate latency to immobility.

Statistics

ANOVAs were conducted to assess the main effects of genotype, rearing condition, and treatment groups, and potential interactions between these variables. Independent t-tests were then run for all significant main effects and interactions. All analyses were conducted using SPSS version 26. Only post-treatment behavioral testing data will be reported.

CHAPTER FOUR

Results

3-Chambered Social Approach

SA was used as a measure of anxiety-like behaviors, with an anxiety-like phenotype indicated by less time spent in the chamber with the other mouse. The main effects of genotype ($F_{(1,122)} = 3.494$, $p = .064$, $\eta^2_p = .026$), rearing condition ($F_{(1,122)} = .451$, $p = .503$, $\eta^2_p = .002$), and treatment ($F_{(1,122)} = 1.217$, $p = .272$, $\eta^2_p = .009$) in this task for time spent in the left zone, the empty chamber, are non-significant, as well as the interactions between them ($F_{(1,122)} = .543$, $p = .462$, $\eta^2_p = .004$, *Figure 3*). The main effects of genotype ($F_{(1,122)} = 3.399$, $p = .068$, $\eta^2_p = .025$), rearing condition ($F_{(1,122)} = .212$, $p = .646$, $\eta^2_p = .002$), treatment ($F_{(1,122)} = 1.921$, $p = .168$, $\eta^2_p = .014$), and the interactions between them ($F_{(1,122)} = .032$, $p = .859$, $\eta^2_p < .001$) for time spent in the right zone, the chamber with the other mouse, are also non-significant, indicating that social interaction behavior was unaffected by treatment, rearing condition, or genotype.

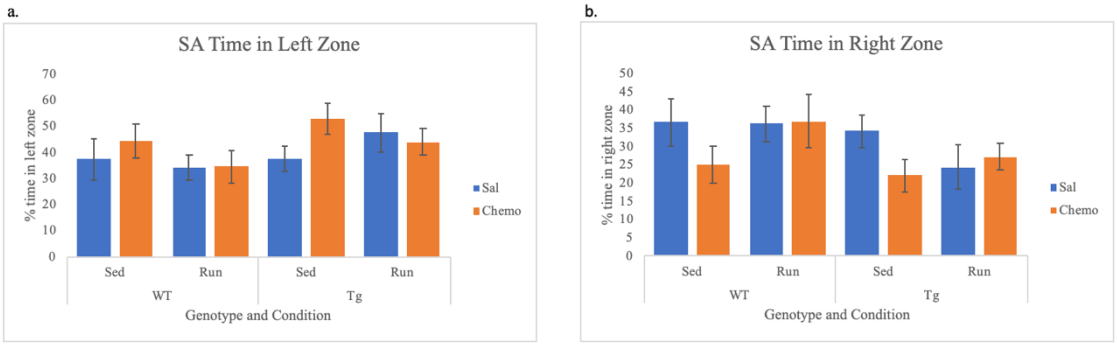

Figure 3. Figure 3a shows amount of time spent in the left zone for each group. Figure 3b shows amount of time spent in the right zone for each group. All are non-significant. Error bar represents standard error of the mean (SEM).

Elevated Plus Maze

EPM was used as a measure of anxiety-like behaviors, with an anxiety-like phenotype indicated by more time spent in closed arms and less time spent in open arms. For the amount of time spent in the open arm, there was a significant interaction effect between genotype, rearing condition, and treatment ($F_{(1,122)} = 4.431$, $p = .037$, $\eta^2_p = .030$, *Figure 4*). Subsequent independent t-tests were run to analyze the difference between groups per genotype, rearing condition, and treatment. There was a significant difference observed between groups WT, Sed, Sal and WT, Sed, Chemo ($t_{(36)} = -2.179$, $p = .036$, $d = .7070$), which means that WT, Sed, Chemo mice spent more time in the open arm than WT, Sed, Sal mice. There was also a significant difference between groups WT, Run, Sal and WT, Run, Chemo ($t_{(39)} = 2.148$, $p = .038$, $d = .0112$), meaning that WT, Run, Sal mice spent more time in the open arm than WT, Run, Chemo mice. There was no significant difference between other groups ($ps > .05$). The main effect of genotype was non-significant ($F_{(1,122)} = .293$, $p = .589$, $\eta^2_p = .002$), the main effect of rearing condition was non-significant ($F_{(1,122)} = .035$, $p = .851$, $\eta^2_p = .000$), and the main effect of treatment was non-significant ($F_{(1,122)} = .003$, $p = .959$, $\eta^2_p = .000$).

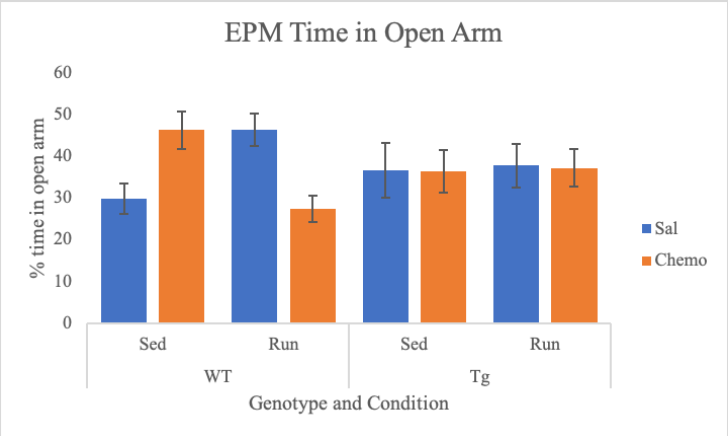

Figure 4. Shows the time spent in the open arm in EPM for each genotype (WT or Tg), rearing condition (Sed or Run), and treatment (Sal or Chemo). Error bars represent SEM.

For the amount of time spent in the closed arm, there was a significant main interaction effect between genotype, rearing condition, and treatment ($F_{(1,122)} = 4.241$, $p = .041$, $\eta^2_p = .029$, *Figure 5*). Subsequent independent t-tests were run to analyze the difference between groups per genotype, rearing condition, and treatment. There was a significant difference between groups WT, Run, Sal and WT, Run, Chemo ($t_{(39)} = -2.174$, $p = .036$, $d = .6903$), meaning that WT, Run, Chemo mice spent more time in the closed arm than the WT, Run, Sal mice. There were no other significant differences between groups ($ps > .05$). The main effect of genotype was non-significant ($F_{(1,122)} = .630$, $p = .429$, $\eta^2_p = .004$), the main effect of rearing condition was non-significant ($F_{(1,122)} = .057$, $p = .812$, $\eta^2_p = .000$), and the main effect of treatment was non-significant ($F_{(1,122)} = .010$, $p = .037$, $\eta^2_p = .030$).

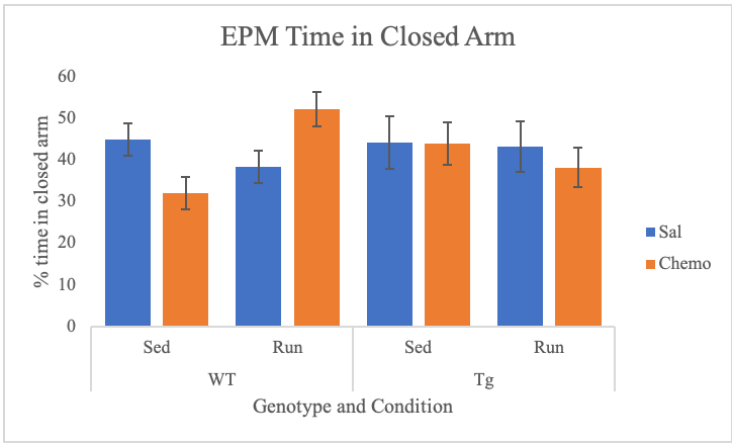

Figure 5. Shows the time spent in the closed arm in EPM for each genotype (WT or Tg), rearing condition (Sed or Run), and treatment (Sal or Chemo). Error bars represent SEM.

Open Field

OF was used as a measure of anxiety-like behaviors, with an anxiety-like phenotype indicated by less time spent exploring and more time spent in periphery. For total distance traveled, there were significant main effects of genotype ($F_{(1, 122)}$ = 19.726, $p < .001$, η^2_p= .120, *Figure 6*) and treatment ($F_{(1, 122)}$ = 8.296, p = .005, η^2_p= .054, *Figure 6*). A subsequent independent t-test was run to analyze differences between groups per genotype and treatment. There was a significant difference between WT, Sed, Sal and Tg, Sed, Sal ($t(35)$ = 2.177, p = .036, d = .7157), indicating that WT, Sed, Sal mice had a higher total distance than Tg, Sed, Sal mice. There was a significant difference between groups WT, Run, Sal and Tg, Run, Sal ($t(42)$ = 3.691, p = .001, d = 1.0531), indicating that WT, Run, Sal mice had a higher total distance than Tg, Run, Sal mice. There was a significant difference between groups WT, Run, Chemo and Tg, Run, Chemo ($t(42)$ = 2.097, p = .043, d = .6885), indicating that WT, Run, Chemo mice had a higher total distance than Tg, Run, Chemo mice. There was a significant difference between groups WT, Run, Sal and Tg, Run, Chemo ($t(41)$ = -2.391, p = .022, d = .1933), indicating that the WT, Run Sal mice had a higher total distance than Tg, Run, Chemo mice. The main effect of rearing condition for total distance was non-significant ($F_{(1, 122)}$ = 3.588, p = .060, η^2_p= .024), meaning that rearing condition did not affect total distance traveled (*Figure 6*). The interactions between genotype, rearing condition, and treatment were also non-significant ($F_{(1,122)}$ = .168, p = .683, η^2_p = .001).

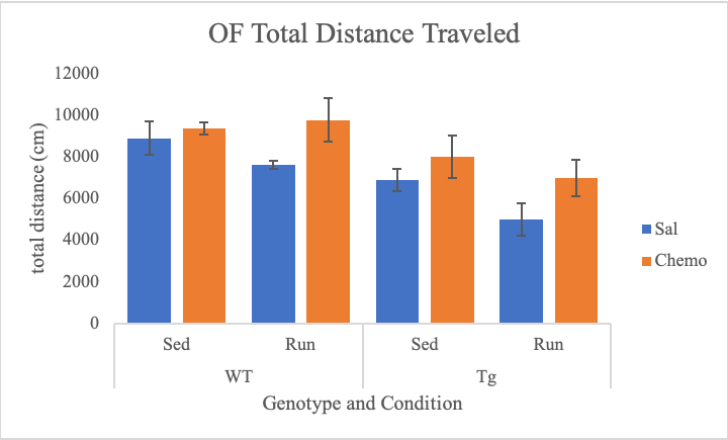

Figure 6. Shows the total distance traveled in OF for each genotype (WT or Tg), rearing condition (Sed or Run), and treatment (Sal or Chemo). Error bars represent SEM.

For time spent in periphery, there was a significant main effect of genotype ($F_{(1, 122)} = 14.558$, $p < .001$, $\eta^2_p = .091$, *Figure 7*). A subsequent independent t-test was run to analyze differences between groups per genotype. There was a significant difference between groups WT, Sed, Sal and Tg, Sed, Sal ($t(35) = -2.624$, $p = .013$, $d = .8075$), indicating that Tg, Sed, Sal mice spent more time in the periphery than WT, Sed, Sal mice. There was also a significant difference between groups WT, Run, Sal and Tg, Run, Sal ($t(42) = -3.213$, $p = .003$, $d = 1.0208$), indicating that Tg, Run, Sal mice spent more time in the periphery than WT, Run, Sal mice. There were no other significant differences between groups. The main effect of rearing condition was non-significant ($F_{(1, 122)} = .097$, $p = .756$, $\eta^2_p = .001$) the main effect of treatment was non-significant ($F_{(1, 122)} = .089$, $p = .766$, $\eta^2_p = .001$), and the interactions between genotype, rearing condition, and treatment was non-significant ($F_{(1,122)} = .178$, $p = .674$, $\eta^2_p = .001$), meaning that rearing condition and treatment had no effect on time spent in periphery (*Figure 7*).

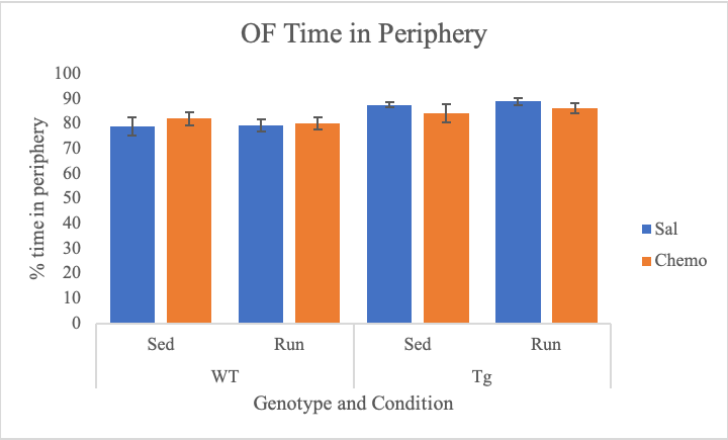

Figure 7. Shows time spent in periphery in OF for genotype (WT or Tg), rearing condition (Sed or Run) and treatment (Sal or Chemo). Error bars represent SEM.

Forced Swim Test

FST was used as a measure of depressive-like behaviors, with a depressive-like phenotype indicated by more time spent immobile. For the percentage of time spent immobile there was a significant main effect of treatment ($F_{(1, 122)} = 4.878$, $p = .029$, $\eta^2_p = .041$, *Figure 8*). A subsequent set of independent t-tests were run to analyze the difference between groups per treatment. There was a significant difference between WT, Sed, Sal and WT, Sed, Chemo ($t_{(37)} = -2.942$, $p = .006$, $d = -.9501$) in which WT, Sed, Chemo mice spent more time immobile than WT, Sed, Sal mice. There was no significant difference between the other groups ($ps > .05$). The main effect of genotype was non-significant ($F_{(1, 122)} = 2.046$, $p = .155$, $\eta^2_p = .017$),the main effect of rearing condition was non-significant ($F_{(1, 122)} = 3.699$, $p = .057$, $\eta^2_p = .031$), and the interactions between genotype, rearing condition, and treatment was non-significant ($F_{(1,122)} = 1.820$, $p = .180$, $\eta^2_p = .016$) (*Figure 8*).

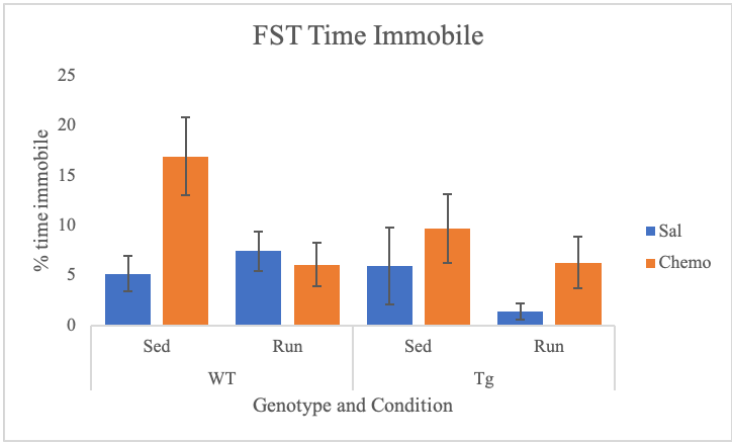

Figure 8. Shows the time spent immobile in FST for genotype (WT or Tg), rearing condition (Sed or Run), and treatment (Sal or Chemo). Error bars represent SEM.

CHAPTER FIVE

Discussion

This study examined affective behavioral changes in mice in regards to three different variables: genotype, rearing condition, and treatment. The behavioral tasks looked for anxiety-like and depressive-like behaviors that were caused by each of these variables and the interactions between them.

While SA showed trends toward social anxiety-like behavior, there were no significant data from this task to claim that genotype, rearing condition, or treatment caused a behavioral change. Generally, the WT, Run, Sal mice spent more time in the chamber with the other mouse while the Tg, Sed, Chemo mice spent more time in the empty chamber, but not to a significant extent.

EPM provided significant interactions between the variables. The interactions showed that WT mice in the sedentary rearing condition were more likely to spend time in the open arm when treated with saline than with chemotherapy, but the WT mice in the run rearing condition were more likely to spend time in the open arm when treated with chemotherapy. It also showed that WT, Run, Chemo mice spent more time in the closed arm than the WT, Run, Sal mice. The results indicate that the chemotherapy treatment induced an anxiety-like behavior in the WT run mice, but it had the opposite effect on the WT Sed mice.

For OF, total distance traveled and time spent in the periphery were examined. The results showed that WT mice traveled more than Tg mice in both rearing conditions and treatments. This indicates that mouse genotype can cause anxiety-like behavior.

Genotype was also significant for time spent in the periphery, meaning that the Tg mice spent more time in the periphery than the WT mice. There were no other significant main effects for OF, suggesting that illness is the biggest contributor in this measure of anxiety-like behavior.

In FST, time spent immobile was used a measure of depressive-like behavior. Treatment was a significant main effect in this task, showing that chemotherapy induced depressive-like behavior in WT sedentary mice. This was not the case for WT mice in the run rearing condition, indicating that exercise may have played a preventative role against the depressive-like behavior. The Sed mice spent more time immobile than the Run mice, although the difference was non-significant. This trend also hints at the protective effect of exercise.

This study shows that chemotherapy treatment may independently cause a depressive-like affect in mice. This is different from Mondie and colleagues (2010) finding no evidence of chemotherapy-induced depressive behavior. It also adds to Walker and colleagues (2017) conclusion by using FST, a better measure of depressive symptoms in mice than NOR or sucrose testing.

In agreeance with Kitamura and colleagues (2015), these data suggest that chemotherapy treatment may also independently cause anxiety-like symptoms in mice. However, it should be noted that genotype was also significant for causing anxiety-like behaviors, indicating that the tumors may be causing it. Another point to consider is the comorbidity of depression and anxiety (Maass et al., 2015). Since the evidence suggests that depressive-like behavior was caused by chemotherapy, the anxiety-like behaviors may also have been a result of that.

Exercise seemed to show protective effects in EPM and FST, which expounds upon previous findings that exercise treats CICI using NMTS and spatial memory tasks (Winocur et al, 2014). However, there were no significant main effects of rearing condition, so the extent to which exercise protects against the development of affective disturbance is still unknown.

Future Directions

This study showed a relationship between breast cancer, chemotherapy, and a sedentary lifestyle with negative affect; however, it did not provide a mechanism. Given the previous studies explored in the literature review, the causes are likely a tumor-induced increase in inflammatory cytokine activity and a chemotherapy-induced decrease in hippocampal neurogenesis (Kitamura et al., 2015, Argyriou et al., 2011, Lee et al., 2004). Future studies should examine cytokine levels and hippocampal neurogenesis after behavioral testing to see if there is a relationship between negative affect and these mechanisms.

It is still unclear whether the anxiety-like symptoms were caused by the tumors, chemotherapy treatment, or as a result of previous negative affect. Future studies should examine the cause of anxiety-like behaviors in the mice in regards to treatment, genotype, and the presence of depressive-like behaviors.

Even though this study examined depressive-like behavior through FST, future studies should use this in addition to another measure of depression. Tail Suspension Test has shown success as a measure of depression in mice and may be used to further indicate the correlation between chemotherapy and depressive-like behaviors (Steru et al., 1985).

The impact of exercise on negative affect remains unclear from this study, although there is some evidence of preventative effects. Future studies should continue to examine exercise as a possible intervention for CICI.

Conclusion

The hypothesis was that chemotherapy would independently cause affective disruption in mice and that exercise would be successful in preventing the disruption. Data from EPM and FST support this hypothesis by showing that chemotherapy induced anxiety-like behavior and depressive-like behavior in these tasks, respectively. Mice that were in the Run rearing condition seemed to be protected from developing negative affect in EPM and FST, which also supports the hypothesis. While this shows that chemotherapy may be inducing anxiety and depression in breast cancer patients, there is also evidence that exercise may help prevent it. Future studies should explore the mechanism behind these results to further understand the cause of the affective disturbance caused by chemotherapy and the effectiveness of exercise in treating it.

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