Chemotherapy Induced Deficits in Cognition and Affective Behavior

DISSERTATION

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Abstract

Advances in cancer detection and treatment have substantially increased the survival rate for breast cancer patients. Chemotherapeutic drugs, particularly anthracyclines, have potentially toxic side effects in the brain- impacting memory, processing time and verbal fluency, depression, and anxiety. More than 30% of patients undergoing chemotherapy for breast cancer suffer from cognitive deficits, depression, or anxiety during and after treatment. Furthermore, for some women these symptoms persist for decades after treatment cessation. Therefore, alleviating anxiety, depression, and cognitive deficits among cancer survivors is important for both quality of life (QOL) and long-term physical health. However, despite the high incidence and significant impact on quality of life, little is known about the cause of the chemotherapy related effects on cognition and affective behavior and no effective treatment currently exists. The present body of work examined the effects of chemotherapeutic agents doxorubicin and cyclophosphamide (AC) on behavior in a non-tumor bearing mouse model. The goals of this dissertation are to determine the physiological mechanism through which AC impairs cognition and affective behavior, as well as test the efficacy of minocycline, an antibiotic with anti-inflammatory properties, in amelioration of chemotherapy-induced changes in behavior. Chemotherapy treated mice exhibited greater anxiogenic-like and depressive-like behavior than the vehicle group; however, there was not a difference in overall activity level, suggesting that these differences were not due to chemotherapy-induced lethargy. In addition, microglia in the brains of chemotherapy-treated mice had
shifted to a proinflammatory state compared to the vehicle group. There was an overall increase in proinflammatory cytokine expression, specifically tumor necrosis alpha (TNF-α) and interleukin 6 (IL-6), in the chemotherapy-treated mice. We also show that administration of minocycline during chemotherapy reduces expression of IL-6 and prevents depression and anxiety behavior in chemotherapy treated mice.

Chemotherapy also affected learning and memory; both the chemotherapy-treated and vehicle-treated mice were able to acquire the task by the final day of training. However, the vehicle-treated mice reached asymptotic performance after fewer training sessions than the chemotherapy-treated mice. Furthermore, the chemotherapy-treated mice took significantly longer to find the escape box than the vehicle-treated mice on training days 2-5. In contrast, there were no group differences in overall locomotor activity level. In addition, chemotherapy increased proinflammatory cytokine expression (TNF-α, IL-1β, and IL-6) in the hippocampus, a region of the brain that is critical for spatial learning and memory. Minocycline reversed the chemotherapy-induced cognitive deficits and increase in IL-6.

Together, these data suggest that the administration of doxorubicin and cyclophosphamide in doses that are at the lower limit of doses used in women being treated for breast cancer, produce neuroinflammation, including microglia activation, increased proinflammatory cytokine expression and concomitant cognitive deficits and alterations in affective behavior in female mice. Treatment with minocycline reverses the behavioral and inflammatory effects of chemotherapy. These findings suggest a possible mechanism by which chemotherapy alters neuropsychological behavior. More
importantly administration of minocycline to cancer patients receiving chemotherapy may improve quality of life and overall physical and mental health; currently no treatments exist for chemotherapy-induced cognitive deficits. Furthermore, minocycline is readily administered in hospitals, is well tolerated over long periods of time, and would be an inexpensive treatment as it is also available in generic form.
Dedication

This thesis is dedicated to my wife, Olivia Olsen Jarrett for being next to me and supporting me every step of the way and to Von and Elaine Jarrett, for guiding and encouraging me.
Acknowledgments

As a masters student at Brigham Young University studying the effects of stress in humans I became familiar with Courtney DeVries research and wanted to work with animal models in her lab. Over the years Courtney has been influential in instructing and helping me to become a scientist. She was always open to new ideas and new projects and her knowledge of animal behavior was essential in helping me design and carry out successful behavioral studies. I will forever be indebted to her for helping me obtain my PhD.

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# Table of Contents

Abstract .................................................................................................................................................. ii

Dedication ............................................................................................................................................... viii

Acknowledgments ....................................................................................................................................... vi

Vita ........................................................................................................................................................ viii

List of Tables .......................................................................................................................................... xi

List of Figures ......................................................................................................................................... xii

Chapter 1: Introduction ........................................................................................................................... 1

Chapter 2: Methods ............................................................................................................................... 86

Chapter 3: Chemotherapy-induced Changes in Affective Behavior ................................................. 97

Chapter 4: Chemotherapy-induced Cognitive Deficits ..................................................................... 132

Chapter 5: Conclusion ........................................................................................................................... 147

References .............................................................................................................................................. 155
List of Tables

Table 1. Self Report of Cognitive Deficits ......................................................... 53
Table 2. Composite Scores for Global Cognitive Function ................................. 55
Table 3. Motor Function ........................................................................................ 57
Table 4. Processing Speed ..................................................................................... 58
Table 5. Visuospatial Ability and Visual Learning and Memory ....................... 61
Table 6. Working Memory and Executive Function ............................................. 63
Table 7. Verbal Ability, Learning, and Memory .................................................. 67
Table 8. Effects of Chemotherapy on Affect in BC Patients ............................... 70
Table 9. Effects of Chemotherapy on Cognition in Rodents ............................... 72
Table 10. Effects of chemotherapy on Affective Behavior in Rodents ............... 74
Table 11. Chemotherapy-induced Inflammatory Changes in Clinical Studies ... 75
Table 12. Chemotherapy-induced Changes in Brain Structure ......................... 77
Table 13. Pre-clinical Mechanistic Studies ......................................................... 80
List of Figures

Figure 3.1. Chemotherapy (72 hours) Increases Protein Expression of TNFα and CCL4 in the Brain ................................................................. 115

Figure 3.2. Chemotherapy Increases mRNA Expression of TNFα and IL-6.... 116

Figure 3.3. Chemotherapy Increases Time Spent Floating in the Forced Swim Test
................................................................................................. 117

Figure 3.4. Chemotherapy Increases Time Spent in EPM Open Arms .......... 118

Figure 3.5. Chemotherapy Does Not Affect Locomotor Activity in BALB/C Mice119

Figure 3.6. Chemotherapy Increases Time Spent Floating in C57bl/6 Mice..... 120

Figure 3.7. Chemotherapy Increases Time Spent in EPM Open Arms in C57bl/6 Mice
................................................................................................. 121

Figure 3.8. Chemotherapy Does Not Affect Locomotor Activity in C57bl/6 Mice122

Figure 3.9. Chemotherapy Increases Microglial Activation Markers in the CNS123

Figure 3.10. Concentrations of AC and Phosphamide Mustard in the Blood and Brain
................................................................................................. 124

Figure 3.11. Minocycline (i.c.v.) Ameliorates Chemotherapy-induced Immobility in the Forced Swim Test ................................................................. 125

Figure 3.12. Minocycline (i.c.v.) Ameliorates Chemotherapy-induced Anxiety-like Behavior in the EPM................................................................. 126
Figure 3.13. Minocycline (i.c.v.) Does Not Affect Overall Locomotor Activity ... 127
Figure 3.14. Minocycline Ameliorates Chemotherapy-induced Gene Expression of Pro-inflammatory Cytokine IL-6 in the Prefrontal Cortex ............................................. 128
Figure 3.15. Minocycline (oral) Ameliorates Chemotherapy-induced Immobility in the Forced Swim Test .................................................................................................................. 129
Figure 3.16. Minocycline (oral) Does Not Affect Overall Locomotor Activity..... 130
Figure 3.17. Minocycline Ameliorates Chemotherapy-induced Increases in Gene Expression of IL-6 .................................................................................................................. 131

Figure 4.1. Chemotherapy Impairs Learning and Memory in the Barnes Maze 141
Figure 4.2. Chemotherapy Increases mRNA Expression of IL-1β and IL-6..... 142
Figure 4.3. Chemotherapy Increases mRNA Expression of TNFα .............. 143
Figure 4.4. Chemotherapy Decreases Dendritic Spine Density in the Hippocampus ........................................................................................................................................... 144
Figure 4.5. Minocycline Ameliorates Chemotherapy-induced Cognitive Impairment ..................................................................................................................................... 145
Figure 4.6. Minocycline Ameliorates Chemotherapy-induced Gene Expression of Pro-inflammatory Cytokine IL-6 in the Prefrontal Cortex ............................................. 146
Chapter 1: Introduction

1. Background
2. Cognitive effects in breast cancer patients
3. Depression and anxiety in breast cancer patients
4. Evidence of Chemotherapy-related Changes in Brain Structure and Function
5. Neuropsychological effects in rodents
6. Mechanism
   6.1 Changes in cytokines in humans
   6.2 Pre-clinical studies
7. Roadblocks
   7.1 Rodent models
   7.2 Drug administration
8. Conclusion

Background

Breast cancer is the most frequently diagnosed cancer in women; one in eight women in the U.S. will be diagnosed with this disease during their lifetime (Howlader N, 2011). Due to improved detection and treatment advances, the overall 5-year and 10-year survival rates for breast cancer (BC) are now 90% and 84.5% respectively (Howlader N, 2011). Thus, there is a growing population of breast cancer survivors for whom persistent side effects of treatment could impinge upon quality of life. The symptoms most commonly reported to reduce quality of life among breast cancer patients include cognitive deficits, depression,
anxiety, fatigue, and sleep disturbance (Bower et al., 2000; Burgess et al., 2005; de Jong, Courten, Abu-Saad, & Schouten, 2002; Fiorentino & Ancoli-Israel, 2006; Myers, 2012). While most studies focused on these symptoms individually, they often occur in clusters (Fiorentino, Rissling, Liu, & Ancoli-Israel, 2012; Ganz et al., 2013; Liu et al., 2009; Rutledge, 2003; So et al., 2009). In a longitudinal study assessing the effects of chemotherapy on fatigue, depression, and sleep disturbance, all symptoms were moderately to strongly correlated from baseline (before chemotherapy) until three weeks after the final treatment, and all women reported impaired sleep, fatigue, and depressive symptoms during treatment compared to baseline (Liu et al., 2009). Furthermore, fatigue, depression, and cognitive impairment worsened across time. Given that fatigue and sleep disruption are among the diagnostic criteria for depression (Fann et al., 2008), it is not surprising that depression is a better predictor of quality of life than either fatigue or insomnia alone (Redeker, Lev, & Ruggiero, 2000). Thus, a larger percentage of the women being treated for BC using chemotherapy develop neuropsychological symptoms that can have a drastic impact on everyday life; the specific focus of this review will be chemotherapy-related deficits on cognitive function and affect.

Chemotherapy-related cognitive impairment (CRCI), often informally referred to as “chemobrain” or “chemofo”, is commonly reported by breast cancer patients (Myers, 2012). The majority of studies with BC patients that incorporate cognitive self-report measures indicate that women who have been
treated with chemotherapy report greater cognitive deficits than BC patients who have not been treated with chemotherapy and matched healthy controls (Table 1). However, the self-reports rarely correlate with objective measures of cognitive deficits. Indeed, results from clinical studies have been largely inconsistent (see Tables 1-7); the effects of chemotherapy on cognition, the percentage of patients affected, and the duration of the cognitive deficits vary greatly across studies. Both individual and experimental factors are likely to contribute to the disparate conclusions among the studies.

In addition to cognitive dysfunction, BC patients often report changes in mood. Depression has received more attention among cancer patients than any other psychiatric disorder (Caplette-Gingras & Savard, 2008) and incidence may be as high as 41% among breast cancer patients (So et al., 2010). Anxiety has received less attention, although it is frequently associated with depression; approximately 45% of breast cancer patients have moderate to high levels of anxiety (Bjorneklett et al., 2012). Depressive symptoms are associated with decreased survival, increased recurrence rates, tumor growth, and decreased quality of life in breast cancer patients, although it is not clear if a causal relationship exists between depression and these outcome measures (Falagas et al., 2007; Fann et al., 2008; Goodwin, Zhang, & Ostir, 2004; Khan, Amatya, Pallant, & Rajapaksa, 2012; Redeker, Lev, & Ruggiero, 2000). However, the effects of chemotherapy on depression and anxiety are understudied and prevalence rates are inconsistent across studies. The inconsistency may be due
to the frequency of self-report measures used to assess these disorders and
difficulty in making a clinical diagnosis of depression in cancer patients with
physical symptoms; some of the physical side effects of chemotherapy and
cancer overlap with DSM-IV criteria for depression, namely lack of energy, poor
concentration, and sleep disturbance (Fann et al., 2008).

While there are many treatment factors and individual susceptibilities that
may increase the risk of cognitive and affective deficits among BC patients,
cognitive decline and depression emerge most consistently and may be the most
debilitating among women whose treatment plan included chemotherapy
(Redeker, Lev, & Ruggiero, 2000; Stewart, Bielajew, Collins, Parkinson, &
Tomiak, 2006). However, the mechanisms through which chemotherapy may be
affecting brain function remains a matter of speculation; to date, no causal
mechanism has been established in either humans or other animals.
Furthermore, there are currently no standard pharmacological treatments for
chemotherapy-induced cognitive dysfunction and treatment of depression using
conventional pharmaceutical methods is limited by potential interference with the
effectiveness of endocrine therapy (ET; Kelly et al., 2010). In sum, the
neuropsychological side effects of chemotherapy tend to be under-recognized
and undertreated, which in turn can compromise quality of life by amplifying the
physical symptoms and functional impairments experienced by the women (Fann
et al., 2008). In addition, untreated depression and concern about side effects
can contribute to poor treatment adherence (Colleoni et al., 2000).
The goals of this review are: 1) to provide a synthesis of the chemotherapy-related side effects on cognition and mood/affective-like behaviors in both human and animal models, 2) provide an overview of the possible data supporting different mechanistic hypotheses, and 3) identify the barriers that have hampered translational research into the causes and potential treatments for the neuropsychological side-effects of chemotherapy.

2. Cognitive effects of chemotherapy in breast cancer patients

Nearly forty years ago the possibility of antineoplastic drugs having neurotoxic side-effects was first reported (Weiss, Walker, & Wiernik, 1974). In 1980, researchers provided evidence in support of this hypothesis by reporting that non-CNS cancer patients being treated with chemotherapy were experiencing cognitive deficits (Silberfarb, Philibert, & Levine, 1980). Since then, the effects of chemotherapy on cognition have garnered much interest and more than a thousand papers on this topic have been published, many in breast cancer patients due to the relatively high survival rate. However, the mechanism by which CRCI occurs remains unknown. Although there is a vast literature discussing the cognitive side effects of chemotherapy in breast cancer patients, the study designs vary drastically, making it difficult to generate cross-study comparisons (Nelson & Suls, 2013; Shilling, Jenkins, & Trapala, 2006; Vardy, Rourke, & Tannock, 2007; Wefel, Vardy, Ahles, & Schagen, 2011). As seen in Tables 1-7, there are many differences across studies, including chemotherapy
agents used, subject age, cognitive instruments, menopause status, treatment duration, concomitant endocrine therapy (ET), and time of testing. Other variations also include race, inclusion criteria, and other treatments. Even within each study there are often differences in chemotherapy agents used, stage of disease, and treatment schedule.

One major issue is the difference in tests used to assess cognitive ability. The studies compiled in Tables 1-7 used more than thirty different cognitive tests to assess cognitive functioning. These assessments are generally broken down into the domains of visuospatial ability, learning, language, attention, working memory, concentration, reaction time, psychomotor speed, and executive functioning. Furthermore, different methods of calculating cognitive scores are used to determine cognitive ability (Shilling, Jenkins, & Trapala, 2006). Shilling (2006) demonstrated the importance of having an appropriate analytical plan by analyzing cognitive impairment in one cohort of breast cancer patients using seven different methods that had been reported in the literature; depending on the method of analysis, cognitive impairment ranged from 12% to 68% in chemotherapy treated patients and 4% to 64% in the healthy controls. In an attempt to encourage standardization across studies and to facilitate consideration of the multitude of individual and treatment factors that can contribute to cognitive deficits in cancer patients, the International Cognition and Cancer Task Force recommended a core set of neuropsychological tests and common criteria for assessing cognitive impairment in cancer patients (Wefel,
Vardy, Ahles, & Schagen, 2011). They recommended prospective, longitudinal trials, including disease specific and healthy control groups (local controls and published normative data), with cognitive assessment at baseline (pre-treatment), and repeat assessment whenever possible to assess changes in cognitive function over time. They also recommend assessing the cognitive domains of learning and memory, processing speed, and executive functioning, using Hopkins Verbal Learning Test-Revised, Trail Making Test, and the Controlled Oral Word Association (COWA) of the Multilingual Aphasia Examination, respectively. These tests were recommended because they assess commonly occurring cancer-related impairments, have adequate psychometric properties, have alternate forms to limit practice effects, and have been translated into multiple languages (Wefel, Vardy, Ahles, & Schagen, 2011).

Study design also has been a major issue in assessing cognitive deficits in BC patients. Until recently, most studies were cross-sectional rather than longitudinal. In fact, meta-analyses published in 2005 and 2006 (Falleti, Sanfilippo, Maruff, Weih, & Phillips, 2005; Stewart, Bielajew, Collins, Parkinson, & Tomiak, 2006) on the cognitive effects of chemotherapy included only one longitudinal study among 11 cross-sectional studies. While the number of longitudinal studies of cognitive deficits in BC patients has increased recently, they still comprised less than half of those incorporated in a recent meta-analysis (8 of 17; Jim et al., 2012) Jim 2012). The advantage of the early cross-sectional studies is that they could be completed in a shorter period of time and at lower
cost than longitudinal studies, while providing an indication of the prevalence of
cognitive deficits among BC patients. An important disadvantage is that cross-
sectional design cannot rule out that cognitive deficits existed among the patients
prior to chemotherapy, nor do they provide information about how cognitive
function changes within an individual in response to treatment and with the
passage of time; indeed, one early cross-sectional study reported that 35% of BC
patients exhibited cognitive deficits prior to chemo treatment when compared to
normative data (Wefel et al., 2004). Importantly, however, the existence of
cognitive deficits prior to systemic treatment, does not rule out a potential
additive or synergistic effect of chemotherapy treatment on cognitive dysfunction.
Several subsequent cross-sectional studies have attempted to address this issue
by including patients who received chemotherapy versus radiotherapy, endocrine
therapy or no additional treatment. Assessing cognitive function six months after
completion of treatment resulted in no cognitive differences between breast
cancer patients treated with both radiotherapy and chemotherapy and those only
receiving radiotherapy (Donovan et al., 2005). Findings from patients assessed
an average of 2 years after treatment did report, however, greater cognitive
impairment in breast cancer patients treated with chemotherapy relative to those
only receiving radiotherapy (Schagen et al., 1999). Results from studies
assessing cognitive functioning five years after treatment with chemotherapy-
versus radiotherapy—also reported conflicting results, one finding chemotherapy
related cognitive impairments (Ahles et al., 2002), and one reporting no
differences (Scherwath et al., 2006). These findings reiterate the importance of performing longitudinal studies, by which cognitive functioning can be assessed before and after chemotherapy treatment for each patient.

In the past decade, numerous longitudinal studies have been published, thus clarifying that many BC patients experience a chemotherapy-related cognitive decline. The vast majority of these longitudinal studies report that breast cancer patients treated with chemotherapy experience a significant decrease in cognitive ability compared to non-chemotherapy treated patient and healthy controls. Findings from three longitudinal studies reported deficits in 11%-31% of patients prior to initiation of chemotherapy (Hermelink et al., 2007; Hurria et al., 2006; Jansen, Dodd, Miaskowski, Dowling, & Kramer, 2008). Two of these three studies, however, reported that even with these deficits at baseline, chemotherapy was associated with a further decline in cognitive function across time (Hurria et al., 2006; Jansen, Dodd, Miaskowski, Dowling, & Kramer, 2008). The third study (Hermelink et al., 2007) found that 27% of the chemotherapy patients experienced a cognitive decline relative to the pre-chemotherapy baseline while 28% demonstrated improvement relative to baseline over the same time period; one drawback of this latter study, however, is that the last session of cognitive testing occurred before the chemotherapy regimen had been completed. This is problematic as others have reported a significant relationship between the number of chemotherapy treatments and degree of cognitive impairment (Hodgson, Hutchinson, Wilson, & Nettelbeck, 2013). Other
longitudinal studies have reported no difference at baseline between chemotherapy treated patients and healthy controls (Bender et al., 2006; Mehlsen, Pedersen, Jensen, & Zachariae, 2009; Schagen, Muller, Boogerd, Mellenbergh, & van Dam, 2006). These findings suggest that while other factors related to cancer may have an effect on cognition, chemotherapy treatment itself has a negative impact on cognitive functioning.

Another crucial issue for BC patients is how long cognitive deficits are likely to persist following completion of chemotherapy. The long-term consequences of chemo on cognition have also been well documented in recent years. While a few studies have suggested that cognitive ability is restored within one year of treatment cessation (Collins, Mackenzie, Stewart, Bielajew, & Verma, 2009), most studies report that cognitive deficits can be detected up to 15 years after the final chemotherapy treatment (Ahles et al., 2002; Bender et al., 2006; Hodgson, Hutchinson, Wilson, & Nettelbeck, 2013; Hurria et al., 2006; Schagen et al., 1999; Shilling, Jenkins, Morris, Deutsch, & Bloomfield, 2005; Wefel, Lenzi, Theriault, Davis, & Meyers, 2004; Yamada, Denburg, Beglinger, & Schultz, 2010). Indeed, a recent study revealed that BC survivors who were treated with chemotherapy 10-15 years prior to testing demonstrated impaired performance on multiple cognitive tests relative to age matched controls without a prior cancer diagnosis (Yamada, Denburg, Beglinger, & Schultz, 2010). Likewise, a similar cognitive deficit was apparent among breast cancer and lymphoma survivors who had received chemotherapy 5-15 years before testing (Ahles et al., 2002).
These studies were cross-sectional, so there is no information available about changes in cognitive ability that may have occurred during the intervening decade and a half, but several longitudinal studies with shorter recovery periods (3-18 months) provide added support for persistent cognitive deficits in at least a subset of BC patients (Ahles et al., 2010; Bender et al., 2006; Jansen, Dodd, Miaskowski, Dowling, & Kramer, 2008; Quesnel, Savard, & Ivers, 2009). Furthermore, a recent meta-analysis concludes that time since treatment is not associated with improvement in cognitive functioning after chemotherapy (Hodgson, Hutchinson, Wilson, & Nettelbeck, 2013). The number of chemotherapy treatments, however, was negatively associated with cognitive performance; more treatments results in poorer cognitive functioning (Hodgson, Hutchinson, Wilson, & Nettelbeck, 2013). This suggests that the duration of chemotherapy treatment may be a better indicator of long term deficits than the time elapsed since completion of chemotherapy. Chemotherapy regimen is also an important factor in determining incidence of cognitive deficits; a high-dose regimen of cyclophosphamide, thiopeta, and carboplatin is nearly four times as likely to result in deterioration in cognitive performance as a standard-dose of 5-fluorouracil, epirubicin, and cyclophosphamide (25% versus 7%, respectively; Schagen, Muller, Boogerd, Mellenbergh, & van Dam, 2006). Similarly, breast cancer patients receiving adriamycin, cyclophosphamide, and fluorouracil had more cognitive complaints than those receiving cyclophosphamide, methotrexate, and fluorouracil (Janelinsins et al., 2012). Thus, the treatment
regimen given is also important factors to consider when assessing the effects of chemotherapy on cognitive functioning.

While it is widely accepted that a decline in cognitive functioning is a possible consequence of chemotherapy in at least a subset of breast cancer patients, the wide variations in study design, cognitive tests, chemotherapy drugs, treatment duration and method of data analysis make it difficult to provide evidence for the mechanisms underlying chemotherapy-induced cognitive deficits and to identify the subset of patients who are likely to be at risk. Studies of both adequate sample size and consistency in variables such as chemotherapy regimen, treatment duration, and data analysis will be instrumental in future research.

3. Depression and anxiety in breast cancer patients

The prevalence of depression in cancer patients has been well studied and ranges from 5% to 50% among breast cancer patients (Caplette-Gingras & Savard, 2008; Knobf, 2007; Massie, 2004; Reece, Chan, Herbert, Gralow, & Fann, 2013). Depression is associated with impairment in quality of life and has a negative influence on compliance of medical treatment, cancer recurrence, recovery, and survival (Colleoni et al., 2000; Falagas et al., 2007; Goodwin, Zhang, & Ostir, 2004; Khan, Amatya, Pallant, & Rajapaksa, 2012; Redeker, Lev, & Ruggiero, 2000). Factors associated with increased affective symptoms among BC patients include younger age, fatigue, pain, insomnia, metastasis, and lack of
Comorbidity of depression and anxiety is common in primary care, with up to 67% of those with a depressive disorder having a comorbid anxiety disorder and 63% with an anxiety disorder also having a current depressive disorder (Hirschfeld, 2001; Lamers et al., 2011). Anxiety is less frequently studied than and often combined with depression as the two are frequently associated. For example, one study reported that nearly 50% of women experienced an episode of depression, anxiety, or both within one year of being diagnosed with breast cancer without reporting the prevalence for depression or anxiety alone (Burgess, 2005). Studies that have reported anxiety levels in breast cancer patients show up to 48% having high levels of anxiety within 3 months of being diagnosed (Boyes et al., 2013; Vahdaninia, Omidvari, & Montazeri, 2010). Anxiety itself is associated with cognitive decline and reduced quality of life (So et al., 2010; Vearncombe et al., 2009). Thus, it is important to assess the effects of anxiety separate from those of depression as well as together.

Relatively few studies have focused specifically on the effects of chemotherapy on depression and anxiety in breast cancer patients, most of them being published within the past 5 years. The prevalence rates vary greatly across studies as there are differences in chemotherapy regimen, treatment duration, testing used and determinant of affective disorder (i.e. cutoff score or DSM-IV
diagnosis). Furthermore, several studies fail to report the specific chemotherapy regimen or separately analyze the drugs to determine if some regimens are more commonly associated with affective disorders (see Table 8). These discrepancies make it difficult to compare across studies; indeed, one group of researchers recently published a systematic review on anxiety in breast cancer patients after concluding that the methodological heterogeneity across studies was too great to support a meta-analysis (Lim, Devi, & Ang, 2011).

As seen in Table 8, not only are there a number of different chemotherapy agents used across studies and within studies, but a number of studies don’t mention the agents used. This is problematic as the specific chemotherapy drug administered may be an important factor influencing the precipitation of depression and anxiety. For example, a recent cross-sectional study reports that treatment with a doxorubicin-based regimen is nearly three times as likely to result in depressive symptoms as a regimen not containing doxorubicin or a non-chemotherapy treatment (Avis et al., 2012). Similarly, a longitudinal study found an increase in depression when treated with doxorubicin/cyclophosphamide, but not other chemotherapy regimens (Reece, Chan, Herbert, Gralow, & Fann, 2013).

While most results suggest that chemotherapy is associated with increased depression and anxiety (Bower et al., 2011; Hwang, Chang, & Park, 2013; Reece, Chan, Herbert, Gralow, & Fann, 2013; Torres et al., 2013), drawing conclusions about the duration of this effect is difficult to do because there are
very few longitudinal studies that extend beyond one year after treatment and include a BC non-chemotherapy control group; a vast majority assessed the effects of an psychological intervention program on affective behavior in patients who all received chemotherapy (Bjorneklett et al., 2012; Kissane et al., 2003; Kissane et al., 2004; Komatsu et al., 2012). However, a recent longitudinal study found that even three years after treatment cessation, chemotherapy treated breast cancer patients had more depressive symptoms than non-chemotherapy treated patients (Hwang, Chang, & Park, 2013). This is in contrast to findings that depression and anxiety generally improve within one year of being diagnosed with breast cancer (Collins, Mackenzie, Stewart, Bielajew, & Verma, 2009; Wefel, Lenzi, Theriault, Davis, & Meyers, 2004); these data suggest that chemotherapy can induce long-lasting effects on depression and anxiety. More long-term longitudinal studies are needed to determine the possible long-term side effects of chemotherapy-related affective disorders on quality of life, recurrence of cancer, and mortality rates. Furthermore, while some patients may improve, not all patients do and how marginal or significant the improvement is yet to be determined. It is also imperative to determine the risk factors for those who do not improve and determine whether chemotherapy treatment is inducing persistent changes in the brain that may precipitate or sustain affective disorders in BC patients.

As briefly discussed above, in the past few years, researchers have found that a number of cancer and chemotherapy-related side effects associated with a
decrease in quality of life frequently occur together in cancer patients. This co-occurrence of symptoms is known as a symptom cluster (Fiorentino, Rissling, Liu, & Ancoli-Israel, 2012). The symptoms most commonly co-occurring are depression, anxiety, fatigue, pain, and trouble sleeping/insomnia (Fiorentino, Rissling, Liu, & Ancoli-Israel, 2012; Liu et al., 2009; Sanford et al., 2013; So et al., 2009). Depression and anxiety are frequently co-morbid in cancer patients (van den Beuken-van Everdingen et al., 2009) and some of the physical side effects of chemotherapy and cancer overlap with DSM-IV criteria for depression, namely lack of energy, poor concentration, and sleep disturbance (Fann et al., 2008). Thus, it is not surprising that these symptoms “cluster” together and that depression is a better predictor of quality of life than fatigue or insomnia alone (Redeker, Lev, & Ruggiero, 2000). However, a recent study found that while depressive symptoms, sleep problems, and fatigue were positively correlated with each other in breast cancer patients, only fatigue was correlated with inflammatory marker tumor necrosis factor receptor two (TNFRII), suggesting that these symptoms may have different underlying biologic mechanisms (Bower et al., 2011).

While some studies suggest that chemotherapy is associated with depression and anxiety, as well as fatigue, pain, and sleep disturbance, in at least a subset of breast cancer patients, the wide variations in study design, affective tests, chemotherapy drugs, and failure to report methodologies makes it difficult to form hypotheses regarding the mechanism of chemotherapy-induced
changes in affect. Longitudinal studies reporting essential study parameters and maintaining consistency in variables such as chemotherapy regimen and treatment duration will be instrumental in future research.

4. Evidence of Chemotherapy-related Changes in Brain Structure and Function

The incorporation of neuroimaging techniques has been instrumental in assessing a neural basis for chemotherapy-related neuropsychological effects. Both structural and functional studies have been performed, using magnetic resonance imaging (MRI) to assess structural differences, and functional MRI (fMRI), positron emission tomography (PET), and electroencephalography (EEG) to assess functional differences.

Structural imaging uses various MRI techniques to determine tissue density or volume differences for the whole brain or a specific region of interest. The first study in a breast cancer population reported that compared to healthy controls, chemotherapy treated patients had increased white matter lesions (Brown et al., 1995). A follow-up, longitudinal study found that these lesions occur shortly after the initiation of chemotherapy, persisting through the one year follow up assessment (Brown et al., 1998). Only four patients completed all assessments and a pre-chemotherapy assessment was not performed; however, these early findings suggested that chemotherapy is associated with structural changes within the brain, highlighting the need for additional research. A similar study, using a larger sample size reported that one year after treatment,
chemotherapy patients had smaller volumes of both gray and white matter in areas related to cognitive processing relative to non-chemotherapy controls (Inagaki et al., 2007); furthermore, volume loss was positively correlated with cognitive deficits in attention and memory. Since then, a number of cross-sectional and longitudinal MRI studies have reported that chemotherapy is associated with changes in gray matter and white matter in various regions of the brain, as well as total brain volume (See table 6; Abraham et al., 2008; de Ruiter et al., 2011; Deprez et al., 2012; Deprez et al., 2011; S. Kesler et al., 2013; Koppelmans et al., 2012; McDonald, Conroy, Ahles, West, & Saykin, 2010). Furthermore, these changes in brain structure are correlated with deficits in attention (Deprez et al., 2011), verbal memory (S. Kesler et al., 2013), executive functioning (S. R. Kesler et al., 2013) and processing speed (Abraham et al., 2008). The imaging studies suggest that these chemotherapeutic-induced changes are widespread rather than localized to a specific brain region, as there were diffuse changes of gray and white matter (Deprez et al., 2012; Koppelmans et al., 2012; Silverman et al., 2007). These changes can be long lasting, as cross-sectional studies reveal that chemotherapy-related changes are detected in patients up to 21 years after cessation of chemotherapy treatment (de Ruiter et al., 2011; S. Kesler et al., 2013; Koppelmans et al., 2012).

Functional imaging is used to measure brain activity, both global and regional. Functional magnetic resonance imaging (fMRI) is a non-invasive technique used to measure brain activity by detecting oxygen changes in blood
volume, the blood oxygen level dependent (BOLD) signal showing regions of high oxygen uptake. BOLD activation in the prefrontal cortex was lower in chemotherapy treated patients than healthy controls during a memory encoding test three years after treatment cessation (Kesler, Bennett, Mahaffey, & Spiegel, 2009). However, BOLD activation was higher in the chemotherapy group across multiple brain regions during a memory recall task; this suggests that chemotherapy may be associated with memory encoding-related functional deficits, and that recalling information is more difficult, thus requiring a greater recruitment of brain regions to perform the given task. Comparing chemotherapy treated patients to non-chemotherapy and healthy controls, findings suggest that both breast cancer and chemotherapy are independently associated with functional changes in the brain (Kesler, Kent, & O'Hara, 2011). Breast cancer also was associated with hypoactivation in the left medial prefrontal and premotor cortex, whereas only the chemotherapy treated group showed reduced activation of the left lateral prefrontal cortex. These results corroborate findings from pre-chemotherapy treatment studies showing that cancer patients have higher level of activation of the inferior frontal gyrus (Cimprich et al., 2010; Scherling, Collins, Mackenzie, Bielajew, & Smith, 2011). These data reiterate the importance of longitudinal studies and non-chemotherapy controls in assessing the effects of chemotherapy on changes in the brain.

Positron emission tomography, a nuclear imaging technique, produces a three-dimensional image by detecting pairs of gamma rays emitted by a tracer
introduced to the body. This technique can be used to assess metabolic activity in the brain. To date only one study has used PET in chemotherapy-related breast cancer studies (Silverman et al., 2007). Chemotherapy patients showed increased activation in the frontal cortex, specifically the inferior frontal gyrus (IFG) and posterior cerebellum, during the short term memory recall task. Furthermore, patients treated with tamoxifen, an estrogen antagonist, in addition to chemotherapeutic agents showed reduced metabolism in the basal ganglia, suggesting a possible effect of hormonal therapy on brain function.

Electroencephalography records electrical activity within the brain by measuring voltage fluctuations from neuronal ionic currents. Only one study has assessed chemotherapeutic-related changes in electrical current in the brain (Schagen, Hamburger, Muller, Boogerd, & van Dam, 2001). While there were no differences for most measures, chemotherapy was associated with asymmetry of alpha rhythm as 41% of patients treated with high dose and 12% treated with standard dose showed alpha rhythm asymmetry compared to 0% of non-chemotherapy controls.

In sum, neuroimaging is a powerful technique that has only recently begun to be used to assess the effects of chemotherapeutic agents on brain structure and function. These findings overwhelmingly support the hypothesis that chemotherapy has detrimental effects within the brain and that these effects may be persistent. Interestingly, the imaging studies have been far more consistent in
documenting cognitive deficits than the neuropsychological studies that do not incorporate imaging.

5. Neuropsychological effects of chemotherapy in rodents

Taken together, the clinical studies in breast cancer patients provide convincing evidence that a significant proportion of women undergoing chemotherapy develop persistent cognitive and affective side effects. Furthermore, the imaging studies suggest that brain structure and function is being affected, even though there is very little evidence that the chemotherapy drugs are passing through the blood brain barrier at high concentrations to directly affect brain cells (Brufsky et al., 2011; Grimm, 2011). However, the fact that there are robust effects on complex behaviors and measureable changes in brain morphology that persist well beyond metabolism of the chemotherapy agents requires that brain function is being either directly or indirectly affected by the chemotherapy drugs. Establishing the physiological mechanism through which chemotherapy is altering brain structure and function can only be accomplished through the use of animal models. Furthermore, this approach allows a systematic and controlled study of the physiological changes in the brain associated with treatment with individual or combined chemotherapeutic agents. Lastly, rodent models allow high throughput testing of potential pharmacological therapies; currently there are no established methods for preventing or treating the cognitive side effects of chemotherapy and some of the most commonly
prescribed selective serotonin reuptake inhibitors (SSRIs)/selective serotonin and norepinephrine reuptake inhibitors (SNRIs) used to treat depression can reduce the effectiveness of tamoxifen in breast cancer patients (Binkhorst et al., 2013; Kelly et al., 2010; Roscoe et al., 2005). Thus, the study of the cognitive and affective consequences of chemotherapy is at a juncture in which improved understanding of the mechanisms underlying chemotherapy’s effects on brain and behavior could facilitate the development of effective treatments.

While there are a wide range of chemotherapeutic agents used to treat cancer patients, most animal models focus on doxorubicin, cyclophosphamide, 5-fluorouracil, or methotrexate. As seen in Table 9, a majority of research has shown that chemotherapeutic agents do indeed induce deleterious cognitive effects in non-tumor models (ElBeltagy et al., 2010; Konat, Kraszpulski, James, Zhang, & Abraham, 2008; Y. Li, V. Vijayanathan, M. E. Gulinello, & P. D. Cole, 2010; Mondie, Vandergrift, Wilson, Gulinello, & Weber; Reiriz et al., 2006; Winocur et al., 2012; M. Yang et al., 2010). A few studies, however, have reported no effects of chemotherapy on cognition (Boyette-Davis & Fuchs, 2009; Fremouw, Fessler, Ferguson, & Burguete, 2012; Gandal, Ehrlichman, Rudnick, & Siegel, 2008; Long et al., 2011; Lyons, Elbeltagy, Bennett, & Wigmore, 2011; Stock, Rosellini, Abrahamsen, McCaffrey, & Ruckdeschel, 1995) while one reported that chemotherapy improved learning (Lee et al., 2006). These contrasting results are likely due to the differences in treatment protocol and tests used for cognitive assessment. For example a single injection of .005mg/kg
methotrexate was administered in one study that failed to detect a chemotherapy effect on cognition (Stock, Rosellini, Abrahamsen, McCaffrey, & Ruckdeschel, 1995), whereas most studies used at least 37 mg/kg and administered multiple injections (Y. Li, V. Vijayanathan, M. Gulinello, & P. D. Cole, 2010; Madhyastha, Somayaji, Rao, Nalini, & Bairy, 2002). Furthermore, three of the studies with negative results used fear conditioning as the cognitive test, which, as discussed later, may not be a good test for assessing chemotherapeutic-related changes on cognitive ability (Fremouw, Fessler, Ferguson, & Burguete, 2012; Gandal, Ehrlichman, Rudnick, & Siegel, 2008; Long et al., 2011). Another study that found no differences used a cognitive test (5CSRTT) not commonly used in these other studies (Boyette-Davis & Fuchs, 2009); in the discussion section the authors acknowledge that this may not be a proper task for this type of study. Thus, it is important to individually assess the drugs and tests used.

Doxorubicin and cyclophosphamide are the most commonly used chemotherapy regimen for the treatment of breast cancer. In rodent models, cyclophosphamide impairs passive avoidance task learning, novel object recognition, and inhibitory avoidance in mice (Reiriz et al., 2006; M. Yang et al., 2010) while doxorubicin impairs inhibitory avoidance in rats (Liedke et al., 2009). Combined, these drugs impair passive avoidance and contextual fear, but not cued fear in female rats (Konat, Kraszpulski, James, Zhang, & Abraham, 2008; Macleod et al., 2007). A few studies found no difference in spatial memory (MWM and NLR), fear conditioning, or novel object recognition (Fremouw,
Fessler, Ferguson, & Burguete, 2012; G. D. Lee et al., 2006; Long et al., 2011; Lyons, Elbeltagy, Bennett, & Wigmore, 2011). However, one study did not perform the tasks until 2-10 months after the last chemotherapy injection (G. D. Lee et al., 2006) compared to most studies testing within one week of treatment (Liedke et al., 2009; Macleod et al., 2007; Reiriz et al., 2006; Winocur et al., 2012). Two of the studies used strains (F344 rats and C57BL/6 mice) not used in the other studies (Fremouw, Fessler, Ferguson, & Burguete, 2012; Long et al., 2011); one of the studies acknowledged that susceptibility to the side effects of chemotherapy may vary by strain (Fremouw, Fessler, Ferguson, & Burguete, 2012). It is also interesting to note that four of the five studies that found impairments used female rats or male mice, while three of the four studies showing no differences using the opposite sex, male rats or female mice.

Methotrexate (MTX) and 5-Fluorouracil (5-FU) are the other two drugs most commonly tested in animal models. Although one study found no effect of MTX+5-FU on contextual fear conditioning or novel object recognition in male mice (Gandal, Ehrlichman, Rudnick, & Siegel, 2008), multiple studies have reported that rats and female mice treated with MTX (alone or with 5-FU) demonstrate cognitive impairments in spatial and visual memory (MWM and NLR), but not cued memory (Fardell, Vardy, Logge, & Johnston, 2010; Y. Li, V. Vijayanathan, M. E. Gulinello, & P. D. Cole, 2010; Madhyastha, Somayaji, Rao, Nalini, & Bairy, 2002; Seiggers et al., 2009; Winocur, Binns, & Tannock, 2011; Winocur et al., 2012; Winocur, Vardy, Binns, Kerr, & Tannock, 2006). Furthermore, the study
finding no difference used mice surgically implanted with electrodes in the hippocampus, which may have caused baseline alterations in behavior (Gandal, Ehrlichman, Rudnick, & Siegel, 2008). Only three studies administered 5-FU alone, two of these reported impairments in spatial memory (NLR) and one in fear conditioning (Lyons, ElBeltagy, Bennett, & Wigmore, 2012; Mustafa, Walker, Bennett, & Wigmore, 2008). The third study (Fremouw, Fessler, Ferguson, & Burguete, 2012) found no differences in fear conditioning or novel object recognition. A possible explanation may be the use of C57bl/6 female mice, the only such study of those found in table 9, as discussed previously. Future studies need to use a wider range of tests in C57bl/6 mice to address this issue. Three other drugs have only been used once each in rodent models, finding cognitive impairments when treated with Oxaliplatin or thioTEPA, but not Paclitaxel. These studies have recapitulated the effects of chemotherapy on various aspects of cognitive functioning in non-tumor rodent models, suggesting that in addition to the effects of cancer, chemotherapeutic agents themselves may be associated with neuropsychological deficits.

A variety of behavioral tests were used to assess cognitive functioning in rodent models. While there are many differences across studies, as seen in table 9, some tasks were more consistently associated with cognitive deficits than other tasks. A majority of studies using passive/inhibitory avoidance, novel location recognition, or Morris water maze tests reported chemotherapy-related impairments (Fardell, Vardy, Logge, & Johnston, 2010; Fardell, Vardy, Shah, &
Johnston, 2012; Konat, Kraszpulski, James, Zhang, & Abraham, 2008; Li et al., 2008; Liedke et al., 2009; Lyons, ElBeltagy, Bennett, & Wigmore, 2012; Madhyastha, Somayaji, Rao, Nalini, & Bairy, 2002; Mondie, Vandergrift, Wilson, Gulinello, & Weber, 2010; Mustafa, Walker, Bennett, & Wigmore, 2008; Reiriz et al., 2006; Winocur, Binns, & Tannock, 2011; Winocur et al., 2012; Winocur, Vardy, Binns, Kerr, & Tannock, 2006; M. Yang et al., 2010; (c.f. Fremouw, Fessler, Ferguson, & Burguete, 2012; Gandal, Ehrlichman, Rudnick, & Siegel, 2008; G. D. Lee et al., 2006; Long et al., 2011; Seigers et al., 2009), whereas most studies using fear conditioning did not (Fremouw, Fessler, Ferguson, & Burguete, 2012; Gandal, Ehrlichman, Rudnick, & Siegel, 2008; G. D. Lee et al., 2006; Long et al., 2011; Seigers et al., 2009), whereas most studies using fear conditioning did not (Fremouw, Fessler, Ferguson, & Burguete, 2012; Gandal, Ehrlichman, Rudnick, & Siegel, 2008; G. D. Lee et al., 2006; Long et al., 2011; Seigers et al., 2009). The novel object recognition test resulted in mixed results (Fardell, Vardy, Logge, & Johnston, 2010; Fardell, Vardy, Shah, & Johnston, 2012; Fremouw, Fessler, Ferguson, & Burguete, 2012; Gandal, Ehrlichman, Rudnick, & Siegel, 2008; Y. Li, V. Vijayanathan, M. E. Gulinello, & P. D. Cole, 2010; Mondie, Vandergrift, Wilson, Gulinello, & Weber, 2010; Seigers et al., 2009; M. Yang et al., 2010). As chemotherapy is associated with some domains of cognitive functioning more than others in breast cancer patients, the same is most likely true in rodent studies, as seen by the different response to chemotherapy across the various test. Furthermore, some tests may be more sensitive to chemotherapy-induced alterations than others. Future studies will be needed to further address this issue.
In keeping with trends in clinical research, most rodent studies of behavioral side effects of chemotherapy have focused on cognitive function; as seen in table 10, very few have assessed effects on affective behavior. Four of the eight studies listed in table 10 used the elevated plus maze (EPM) to assess anxiety-like behavior after administering doxorubicin. Three of the four found that chemotherapy did induce anxiety-like behavior, the fourth (Kujjo et al., 2011) only analyzed risk-assessment behavior, instead of the commonly reported time spent in open arms (Wall & Messier, 2001). Only one study assessed the anxiogenic effects of cyclophosphamide (Reiriz et al., 2006), reporting no drug effect. However, this study used open field to assess anxiety-like behavior, but did not provide the data or include sufficient details regarding the parameters used to make an independent judgment. Two studies administering MTX reported conflicting results; one study reported increased freezing during fear conditioning (Gandal, Ehrlichman, Rudnick, & Siegel, 2008) while the other found no difference using the light/dark box (Madhyastha, Somayaji, Rao, Nalini, & Bairy, 2002). Experimental designs were not ideal for these studies, however, as fear conditioning is a test used for learning and memory and mice in the second study were allowed 4 days of acclimation in the light/dark box although this test is generally used without acclimation (Hascoet & Bourin, 2009). Only two studies assessed depressive-like behavior (Kujjo et al., 2011; Mondie, Vandergrift, Wilson, Gulinello, & Weber, 2010). Doxorubicin was shown to increase depressive-like behavior in the forced swim but not tail suspension test (Kujjo et
al., 2011), whereas thioTEPA did not increase depressive-like behavior in the forced swim or tail suspension tests (Mondie, Vandergrift, Wilson, Gulinello, & Weber, 2010).

While very few studies have assessed the effects of chemotherapy on affective behavior in rodent models, the findings suggest that chemotherapy does induce anxiogenic, and possible depressive-like, behavior in rodents. However, only one study attempted to reverse the chemotherapy-induced effects on affective behavior, finding that alprazolam (a benzodiazepine) reversed the anxiety-like behavior associated with doxorubicin (Anwar, Pillai, Khanam, Akhtar, & Vohora, 2011). Similarly numerous studies have assessed the mechanistic effects of chemotherapy in animal models without testing behavioral outcomes, as discussed in the next section. It is imperative that future studies address the mechanism and behavior together and attempt to reverse the cellular and behavioral changes induced by chemotherapy.

6. Mechanism

It has long been hypothesized that chemotherapy agents may have neuropsychological effects in non-CNS tumor cancer patients. Over the past decade findings from longitudinal studies in breast cancer patients and non-tumor animal models have provided support for these hypotheses, as previously discussed. However, not all patients receiving chemotherapy experience these side effects, with incidence rates ranging from 15%-80% (Ahles et al., 2010;
Furthermore, these side effects are transient for some patients, while for others they can persist for more than a decade (Yamada, Denburg, Beglinger, & Schultz, 2010). In recent years, a growing number of clinical and pre-clinical studies have attempted to assess the cause by which chemotherapy induces neurological deficits. Although the mechanism has not been elucidated, clinical studies utilizing imaging techniques and peripheral immune measures, in addition to pre-clinical studies (in-vivo and in-vitro), have identified specific effects and potential biological mechanisms by which chemotherapy is hypothesized to hamper neuropsychological functioning.

6.1 Immune-signaling in clinical studies

Recent literature suggests that people suffering with mild cognitive impairment have elevated levels of circulating pro-inflammatory cytokines (Alvarez, Cacabelos, Sanpedro, Garcia-Fantini, & Aleixandre, 2007; Magaki, Mueller, Dickson, & Kirsch, 2007) and that this pro-inflammatory bias may be associated with cognitive deficits in cancer patients (Ahles & Saykin, 2007; Cleeland et al., 2003; Penson et al., 2000; Wang et al., 2010). Furthermore, some chemotherapy agents induce a peripheral pro-inflammatory response in an animal model (Tangpong et al., 2006), thereby providing support for the hypothesis that chemotherapy-induced elevation in inflammatory markers may play a role in neuropsychological deficits following chemotherapy treatment.
Indeed, the side effects of immunotherapy, treatment with pro-inflammatory cytokines, include cognitive deficits, depression, and fatigue (Trask, Esper, Riba, & Redman, 2000). Although few clinical studies have assessed the immune response to chemotherapy treatment in breast cancer patients, findings overwhelmingly suggest that patients receiving chemotherapy do indeed express higher levels of certain peripheral pro-inflammatory markers than in BC patients who do not receive chemotherapy and healthy controls (Table 11). Increases in circulating interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α), including its receptor soluble TNF receptor type 2 (sTNFrII) are most commonly associated with chemotherapy (Janelinsins et al., 2012; Pusztai et al., 2004; Torres et al., 2013; Tsavaris, Kosmas, Vadiaka, Kanelopoulos, & Boulamatsis, 2002). In addition to assessing chemotherapy-related changes in cytokine expression, recent studies have also assessed functional outcomes related to these changes in cytokine levels (Bower et al., 2013; S. Kesler et al., 2013). Compared to healthy controls, breast cancer patients had higher levels of TNF-α and IL-6, memory impairment, and reduced hippocampal volume in the left hemisphere (S. Kesler et al., 2013); a chemotherapy-related reduction in size of the right hippocampus approached significance (p=.07). Furthermore, total memory functioning (Hopkins Verbal Learning Test (HVLT) total) and hippocampal volume was associated with TNF-α and IL-6 in the chemotherapy group, but not the healthy controls. Although this study did not include a non-chemotherapy breast cancer control group, they did report that TNF-α concentrations decreased
as time since the final chemotherapy treatment increased (S. Kesler et al., 2013). Furthermore, there was no relationship between stage of breast cancer and soluble TNF-α concentrations, suggesting that these changes are related to chemotherapy treatment and not the cancer (S. Kesler et al., 2013). Together, these data suggest that chemotherapy-induced cognitive deficits and changes in brain structure may be related to chemotherapy-related changes in TNF-α and IL-6.

A longitudinal study using a BC control group that did not receive chemotherapy reported higher levels of soluble TNF-α receptor type 2 (sTNF-RII) in the chemotherapy group shortly after, and 6 months following, treatment with no difference 1 year later (Ganz et al., 2013). PET imaging also revealed that sTNF-RII is associated with diminished metabolism in the inferior frontal cortex and memory complaints in the chemotherapy group, but not the control group. The longitudinal decline in sTNF-RII was also correlated with fewer memory complaints over twelve months. Although there was no relationship between cytokines and non-self-reported cognitive functioning, the neuropsychological outcomes have not been assessed beyond baseline as the full study has not yet been completed. A third study, also incorporating non-chemotherapy breast cancer controls reported that chemotherapy, but not radiotherapy, was associated with higher levels of NF-kB DNA binding in peripheral blood mononuclear cells, IL-6, and sTNF-R2, as well as depression and fatigue (Torres
et al., 2013). Chemotherapy and NF-kB DNA binding were also significant predictors of depression.

While these findings support an inflammatory based mechanism by which chemotherapy alters neuropsychological functioning, more research is needed to assess a long-term presence and influences of these cytokines and to determine if a causal relationship exists between chemotherapy-induced inflammation and changes in brain structure and function. Only two longitudinal studies have assessed cytokine concentrations out to one year after treatment, both finding a decrease in sTNFRII across time (Ganz et al., 2013; Pomykala et al., 2013), while C-reactive protein (CRP) remained higher in chemotherapy treated patients in one study (Pomykala et al., 2013), the other finding no treatment differences in CRP at any time point (Ganz et al., 2013). One study also suggested that findings may be drug specific; concentrations of IL-6 were higher in breast cancer patients treated with AC/CAF compared to CMF (Janelinsins et al., 2012). This may be due to the anti-inflammatory properties of methotrexate (Johnston, Gudjonsson, Sigmundsdottir, Ludviksson, & Valdimarsson, 2005). However, removing the participants who had received methotrexate did not alter the results in a similar study (S. Kesler et al., 2013). These limited findings suggest that chemotherapy-induced inflammation is correlated with neuropsychological effects seen in breast cancer patients treated with chemotherapy agents.

6.2 Pre-clinical studies
Findings from numerous clinical studies suggest that various chemotherapy agents are associated with neuropsychological deficits. Furthermore, imaging studies have found that patients treated with chemotherapy have structural and functional changes in their brains compared to healthy controls as well as non-chemotherapy treated breast cancer patients. Pre-clinical studies corroborate and extend these findings, reporting chemotherapy-induced neuropsychological changes in non-tumor bearing rodents. However, even with this vast amount of literature, the mechanism by which this occurs remains unknown. Consistent results in pre-clinical and clinical research suggest that neurobiological research in animal models may be useful in delineating the mechanisms through which chemotherapy alters behavior. In the past decade, a growing number of pre-clinical studies have proposed potential mechanisms associated with chemotherapeutic neurotoxicity within the CNS, but no interventional studies have attempted to establish causation.

As described above, a wide range of chemotherapeutic agents have been linked to cytotoxic damage within the CNS (see Table 13), including doxorubicin, cyclophosphamide, methotrexate, 5-fluorouracil, paclitaxel, thioTEPA, carmustine, cytarabine, cisplatin, ifosfamide, temozolomide, vinblastin, cytosine arabinoside, and vincristine (Dietrich, Han, Yang, Mayer-Proschel, & Noble, 2006; Eijkenboom & Van Der Staay, 1999; Han et al., 2008; Janelinsins et al., 2010; Koros & Kitraki, 2009; Rzeski et al., 2004; Tangpong et al., 2006; M. Yang
et al., 2011). Furthermore, these drugs alter a variety of biological components within the brain including neurogenesis, cell death, oxidative stress, inflammation, neurotransmitters and monoamines (Aluise et al., 2011; Aluise et al., 2010; Briones & Woods, 2011; Han et al., 2008; Madhyastha, Somayaji, Rao, Nalini, & Bairy, 2002; Tangpong et al., 2006).

Doxorubicin has been shown to increase CNS inflammation, cell death, apoptosis, oxidative stress and impair mitochondrial functioning (Joshi et al., 2007; Kang, Chess-Williams, Anoopkumar-Dukie, & McDermott, 2013; Oz & Ilhan, 2006; Tangpong et al., 2011). Rodents treated with doxorubicin had higher concentrations of pro-inflammatory cytokine TNF-α, increased levels of inducible nitric oxide synthase (iNOS) mRNA and more oxidative stress as indicated by increased protein and lipid oxidation and decreased glutathione (GSH; Aluise et al., 2011; Joshi et al., 2007; Tangpong et al., 2007; Tangpong et al., 2006).

Administration of an antioxidant, mercaptoethane sulfonate (MENSA), prevented the changes in TNF-α and oxidative stress (Aluise et al., 2011). The increase in TNF-α may be associated with mitochondrial dysfunction via oxidative stress as doxorubicin increased TNF-α in wild-type and iNOS knock-out mice, but only affected mitochondrial respiration in the WT mice (Tangpong et al., 2007). Furthermore, inhibiting TNF-α ameliorated the increase in iNOS mRNA and mitochondrial dysfunction (Tangpong et al., 2006). Future studies using doxorubicin need to assess behavioral outcomes in addition to oxidative stress and inflammation to validate the biological relevance of these findings.
Cyclophosphamide is associated with alterations in neurogenesis, oxidative stress, cell death, and histone acetylation (Briones & Woods, 2011; Manda & Bhatia, 2003; Rzeski et al., 2004; M. Yang et al., 2010). Rodents treated with cyclophosphamide showed a decrease in neurogenesis which was also associated with cognitive impairment in the novel object recognition, novel location recognition, Morris water maze, and fear conditioning tests (Briones & Woods, 2011; Christie et al., 2012; Janelinsins et al., 2010; M. Yang et al., 2010). Similar to doxorubicin, cyclophosphamide treatment resulted in decreased levels of glutathione and glutathione peroxidase and increased levels of malondialdehyde, aspartate aminotransferase, and alanini aminotransferase, indicative of oxidative stress (Bhatia, Manda, Patni, & Sharma, 2006; Manda & Bhatia, 2003; Oboh & Ogunruku, 2010). Although these studies assessing cyclophosphamide associated oxidative stress did not attempt to test neuropsychological deficits, a study assessing oxidative stress and neuropsychological outcome reported that treatment with methotrexate increased oxidative stress, depressive-like behavior, and cognitive dysfunction. Thus, chemotherapy-associated increases in oxidative stress may have an effect on neuropsychological functioning. Cyclophosphamide-treated rats also had histone modifications in the hippocampus and prefrontal cortex, but not striatum, relative to control rats (Briones & Woods, 2011). Treatment resulted in increased acetylation of histone H3 and decreased histone deacetylation activity,
suggesting that cyclophosphamide may influence epigenetic modifications within the brain.

As seen in Table 13, oxidative stress is commonly studied in and associated with doxorubicin and cyclophosphamide and could be one mechanism contributing to chemotherapy-related cognitive impairment (Ahles & Saykin, 2007). Oxidative stress is a common source of DNA damage that occurs due to secondary effects of metabolism and exogenous toxins (Fishel, Vasko, & Kelley, 2007). Doxorubicin and cyclophosphamide are effective cancer treatments, in part because they disrupt the DNA. These effects are not cell specific, however, and cause similar damage to other cells. As chemotherapy is associated with cognitive deficits, similarly, DNA damage is higher in elderly patients diagnosed with mild cognitive impairment compared to healthy controls (Keller et al., 2005). These findings suggest that oxidative stress is a logical mechanism for chemotherapy induced neuropsychological changes and warrants further research.

Pre-clinical studies using methotrexate have found this drug to be associated with a wide range of alterations in the brain including neurogenesis, gliogenesis, oxidative stress, apoptosis, blood vessel density, CSF composition, electrophysiology, and neurotransmitters (Gandal, Ehrlichman, Rudnick, & Siegel, 2008; Madhyastha, Somayaji, Rao, Nalini, & Bairy, 2002; Rajamani, Muthuvel, Senthilvelan, & Sheeladevi, 2006; Seigers et al., 2008; Silverstein & Johnston, 1986; Vijayanathan, Gulinello, Ali, & Cole, 2011; M. Yang et al., 2012;
Methotrexate also is associated with suppressed neurogenesis and gliogenesis of the hippocampus, specifically the subgranular zone, and related deficits in learning and memory (Lyons et al., 2011; Seigers et al., 2010; Seigers et al., 2008; Vijayanathan, Gulinello, Ali, & Cole, 2011; M. Yang et al., 2012; M. Yang et al., 2011). Interestingly, in vitro studies suggest that susceptibility to methotrexate induced cytotoxicity or apoptosis depends on the maturity of the cells; cytotoxicity of immature hippocampal cells increases in a dose-dependent manner whereas mature cells do not change. Furthermore, immature cells also have increased expression of active caspase-3 and cleaved poly (ADP-ribose) polymerase (PARP), markers of apoptosis (M. Yang et al., 2011). This same study reported that pretreatment with a caspase-3 inhibitor blocked methotrexate-induced hippocampal cell death, suggesting that methotrexate may induce apoptosis of immature hippocampal cells.

Methotrexate is also associated with changes in cerebral spinal fluid (CSF) components. Folate is a B vitamin found in the blood and CSF that is essential for brain development and optimal cognitive functioning. It is also involved in neurotransmitter synthesis and metabolism of homocysteine, a neurotoxic amino acid (Y. Li, V. Vijayanathan, M. Gulinello, & P. D. Cole, 2010). Low circulating folate is associated with mild cognitive impairment and depression (Bell et al., 1992; Riggs, Spiro, Tucker, & Rush, 1996). In pre-clinical studies MTX administration, both acute and chronic, was associated with reduced folate in the CSF and serum (Y. Li, V. Vijayanathan, M. Gulinello, & P.
Methotrexate is also associated with reduction of monoamines including dopamine, norepinephrine, and serotonin (Madhyastha, Somayaji, Rao, Nalini, & Bairy, 2002; Silverstein & Johnston, 1986). These are important findings as these monoamines play critical roles in depression and anxiety (Dunlop & Nemeroff, 2007). Methotrexate is involved in a number of different possible mechanisms underlying neuropsychological deficits. Future research needs to address whether these different components are related to or independent of each other.

Although less extensively studied, 5-FU, thioTEPA, cytosine arabinoside, carmustine, cisplatin, and cytarabine are also associated with cytotoxicity in the brain (Dietrich, Han, Yang, Mayer-Proschel, & Noble, 2006; Han et al., 2008; Mondie, Vandergrift, Wilson, Gulinello, & Weber, 2010). 5-FU is associated with reduced hippocampal cell proliferation and impaired cognitive functioning (NLR; ElBeltagy et al., 2010; Lyons, ElBeltagy, Bennett, & Wigmore, 2012; Mustafa, Walker, Bennett, & Wigmore, 2008). Fluoxetine, an anti-depressant, improved the treatment-associated behavioral and proliferation deficits (ElBeltagy et al., 2010; Lyons, ElBeltagy, Bennett, & Wigmore, 2012). Findings from an in vitro study suggest that CNS cells are more susceptible to chemotherapy agents than cancer cells (Han et al., 2008); chemotherapeutic agents are known to inhibit proliferation, however, treatment of 5-FU killed a majority of CNS cells in vitro without harming tumor cells. Both mature oligodendrocytes and their precursors, essential for myelinating axons, were destroyed. In vivo, chemotherapy caused a
2.5 fold increase in apoptosis in the subventricular zone and a four-fold increase in apoptosis in the dentate gyrus of the hippocampus one day after treatment, with increased cell death continuing for two weeks after treatment with 5-FU (Han et al., 2008). Furthermore, chemotherapy treatment caused delayed abnormalities in transcriptional regulation and maintenance of myelin integrity, causing myelin loss months after treatment ended (Han et al., 2008). Carmustine, cisplatin, and cytarabine had similar effects, being more toxic for CNS progenitor cells and non-dividing oligodendrocytes than cancer cells, with cell death increasing for weeks after treatment cessation (Dietrich, Han, Yang, Mayer-Proschel, & Noble, 2006). These findings offer a possible explanation for the long-term neuropsychological effects of chemotherapeutic agents.

Pre-clinical studies have been able to reproduce the neuropsychological effects of chemotherapy seen in cancer patients and assess the effects of chemotherapeutic agents individually, whereas a majority of clinical studies involve multiple chemotherapeutic agents. Although the mechanism has yet to be elucidated, findings from pre-clinical studies clearly show possible components that may be involved including neurogenesis/gliogenesis, apoptosis, inflammation, oxidative stress, CSF composition, and monoamines. Potential interventions have also been suggested including administration of anti-oxidants (Aluise et al., 2011; Oboh, Akomolafe, Adefegha, & Adetuyi, 2012; Tangpong et al., 2007), stimulating neurogenesis (Janelsin et al., 2010), and anti-depressant Fluoxetine (ElBeltagy et al., 2010). However, these interventions have not yet
been used in a tumor-bearing model, which may result in a different outcome. It is also difficult to determine which mechanistic components have a direct versus indirect effect on neuropsychological ability and the extent to which they are inter-related. Furthermore, pre-clinical research has yet to address the observation that impairment only occurs in a subset of patients. Future studies will need to address this issue, looking at possible genetic variances including drug resistance pumps, DNA repair mechanisms and cytokine/hormone production and receptors (Ahles & Saykin, 2007).

7. Pre-clinical Roadblocks

Many cancer patients treated with chemotherapeutic agents experience neuropsychological deficits, with reported incidences of 15% to 80% (Ahles et al., 2010; Bjorneklett et al., 2012; Koppelmans et al., 2012; So et al., 2010; Wefel, Saleeba, Buzdar, & Meyers, 2010). Although numerous clinical studies have assessed the relationship between chemotherapeutic agents and neuropsychological deficits, as previously discussed, a mechanism has not been elucidated. In the past decade, a growing number of rodent models have emerged, greatly increasing our understanding of possible key components involved in this relationship. Most importantly, animal models were able to recapitulate the chemotherapy related neuropsychological deficits reported in clinical studies. However, basic research in this area began only recently and is still in the early stages. Future studies will need to be more mechanistic and
focus more on the cellular and molecular components and pathways involved. Multiple challenges or roadblocks in animal models emerge as there are experimental design differences across studies that may result in various findings, in turn hindering translational outcomes for cancer patients. These challenges are discussed below.

7.1 Rodent models

When designing a translational study, it is important to determine the degree of translational relevance related to a given model. Rodent models vary greatly in areas that may hinder their translational value to cancer patients, such as age, sex, and hormone concentrations. A majority of breast cancer patients are 50 years old or older, only five percent being younger than 40 ("Breast Cancer Facts & Figures 2011-2012"). However, most pre-clinical research uses young adult rodents (see Table 13). Clinical studies suggest that age is associated with decreased cognitive ability (Verhaeghen & Salthouse, 1997). Furthermore, pre-clinical studies report age-related changes in the brain that are associated with increased inflammation and depressive-like behavior (Corona, Fenn, & Godbout). In order to better mimic the clinical breast cancer population, thus potentially having more translational relevance, future studies should examine whether the central and behavioral effects of chemotherapy differ in young adult and aged adult mice.
Choosing the appropriate sex is important in pre-clinical research as sex differences exist in both immune function and cognitive ability (Jonasson, 2005; Klein & Nelson, 1998; Nava-Castro, Hernandez-Bello, Muniz-Hernandez, Camacho-Arroyo, & Morales-Montor, 2012). Males generally have reduced immune responses compared to females, likely due to the testosterone suppressing the immune system (Nava-Castro, Hernandez-Bello, Muniz-Hernandez, Camacho-Arroyo, & Morales-Montor, 2012; Olsen & Kovacs, 1996). Indeed, male mice had a higher immune response after infection if their gonads had been removed compared to those with intact gonads (Kittas & Henry, 1980). Similarly, male rats are more susceptible to Adriamycin-induced nephropathy than females and castrated males were less susceptible than sham-operated controls (V. W. Lee & Harris, 2011). Sex differences also exist in cognitive functioning in rodents. A meta-analysis reported that male rats performed better than females in the water maze and radial arm maze, tasks assessing spatial, working, and reference memory (Jonasson, 2005). In contrast, female mice performed better in the water maze than male mice, while male mice still performed better than female mice in the radial maze. These findings suggest that results may vary across studies based on the species and sex of animal used as immune function and cognitive ability vary across species and sex. Furthermore, when choosing which sex to use, it is important to determine the desired translational goal of the study. As breast cancer is the second most common form of cancer, is female dominant, and has a high survival rate, most
clinical research has used breast cancer patients when assessing the side-effects of chemotherapy (American Cancer Society. Cancer Facts & Figures 2013. Atlant: American Cancer Society; 2013, 2013). Thus, it would be most beneficial to use female animal models for translational research in future studies. It is essential that these aspects are accounted for and understood before beginning an experiment, as the species and sex of rodent used may be just as important as choosing which chemotherapeutic agent or behavioral test to use and will have varying effects on translational outcomes.

The effect of hormones on cognitive functioning is also important in animal models as clinical studies suggest that changes in hormone levels and menopausal status may alter cognitive ability (Berent-Spillson et al.; Jenkins, Shilling, Fallowfield, Howell, & Hutton, 2004). Animal models assessing the neuropsychological effects of chemotherapy vary in this regard as some use ovariectomized females to better mimic post-menopausal breast cancer patients (Macleod et al., 2007), while others leave the ovaries intact (Winocur, Vardy, Binns, Kerr, & Tannock, 2006). To our knowledge no pre-clinical chemotherapy studies have assessed neuropsychological functioning comparing ovariectomized and non-ovariectomized rodents. This would be beneficial in determining if the effects of specific chemotherapy agents vary according to hormones or if hormone levels, one way or the other, exacerbates the effects of chemotherapy on neuropsychological outcomes. As previously mentioned, assessing the desired translational relevance is important when designing an
animal model. A majority of breast cancer patients are peri- or post-menopausal before beginning chemotherapy treatment (Ahles et al., 2010; Collins, MacKenzie, Tasca, Scherling, & Smith, 2012; Del Mastro et al., 2006; Jenkins et al., 2006; Shilling, Jenkins, Morris, Deutsch, & Bloomfield, 2005), less than 25% being pre-menopausal (Bines, Oleske, & Cobleigh, 1996; Hankey, Miller, Curtis, & Kosary, 1994). Furthermore, chemotherapeutic agents frequently induce early menopause in premenopausal breast cancer patients (Bines, Oleske, & Cobleigh, 1996; Del Mastro et al., 2006). As the main goal of related pre-clinical research is to improve the quality of life in chemotherapy treated patients by suppressing treatment-related neuropsychological deficits, using ovariectomized females would be most beneficial in translational outcomes.

A number of different strains of rats and mice have been used to study the mechanism of chemotherapy-related neuropsychological deficits including BalbC, C57bl/6, Swiss albino, ICR, and B6C3 mice, and Wistar, Lister-hooded, Sprague-Dawley, athymic nude, Buffalo, and Long Evans rats. Although these species and strains are similar in many instances and have all shown deficits related to chemotherapeutic agents, there are differences among them that may result in different outcomes in future chemotherapy studies. For example, differences in neurogenesis are seen between rats and mice as newly forming hippocampal neurons are more numerous, faster-maturing, and more likely to be recruited to learning circuits in rats than in mice; contributions of these new neurons to fear memory are also greater in rats than mice (Snyder et al., 2009).
Whether, chemotherapy has similar effects on neurogenesis in rats and mice remains to be determined.

Another important factor could be the immune system, as it is hypothesized to play a role in chemotherapeutic side effects (Kang, Chess-Williams, Anoopkumar-Dukie, & McDermott, 2013; Tangpong et al., 2007) and variations in immune response exist between strains (Griffin & Whitacre, 1991; Palumbo et al., 2010; I. V. Yang et al., 2009) and species of rodents (Yang, 2009). At basal levels Lewis rats have higher mononuclear cell counts and CD4 lymphocytes than Fischer (F344) rats (Griffin & Whitacre, 1991). Furthermore, stress induces different immune responses across strains, generating a Th2 response in BALB/c and Th1 response in C57BL/6 mice; these responses were marked by a difference in cytokine expression (Palumbo et al., 2010). For example, BALB/c mice have increased IL-4 and IL-10 and decreased INF-γ concentrations compared to C57BL/6 mice (Palumbo et al., 2010). Similarly, treating sixteen different mouse strains with LPS for 6 hours resulted in a wide range of cytokine concentrations depending upon the strain (I. V. Yang et al., 2009). Differences were also apparent between rats and mice with cells from the small intestine of C57BL/6 mice having higher concentrations of TNF-α, IL-4, and GM-CSF, whereas Sprague-Dawley rats had higher concentrations of IL-1b (I. V. Yang et al., 2009). These differences in immune regulation could potentially result in chemotherapeutic agents having differing effects across species and strains. Indeed, in assessing adriamycin-induced nephropathy, C57BL/6 mice
were much more resistant to renal injury than BALB/c mice, needing a higher dose to provoke injury (V. W. Lee & Harris, 2011).

As seen in Table 13 most pre-clinical research has assessed biological and/or behavioral outcome in a non-tumor model using a single chemotherapeutic agent. Using non-tumor models has been beneficial in assessing the effect of chemotherapy on neuropsychological functioning independent of cancer and will continue to be paramount in elucidating the mechanism. However, as therapeutic measurements arise it will be essential to assess these treatments in a tumor-bearing model as it is well documented that cancer alters immune functioning and behavior (Wefel et al., 2004; M. Yang et al., 2010). Furthermore, tumor models in which the tumor is removed before administering chemotherapy will also be essential, as this models clinical adjuvant treatment regimens. Similarly, assessing the effects of individual chemotherapeutic agents has been and will be essential to understanding the mechanistic relationship between these drugs and related neuropsychological deficits. However, as cancer patients are frequently treated with multiple drugs, some pre-clinical experiments will also need to include these combined cocktails to assess the possible effect of drug interactions on cognition and behavior.

7.2 Drug Administration

Chemotherapeutic agents in breast cancer patients are commonly administered intravenously, rapidly circulating throughout the body. Each
treatment generally consists of multiple cycles administered every few weeks. In clinical studies, dosage and number of treatment cycles is positively correlated with neuropsychological deficits (Hodgson, Hutchinson, Wilson, & Nettelbeck, 2013). As it is difficult to mimic the slower drip-line administration of chemotherapeutic agents seen in cancer patients, rodent models generally administer a bolus injection intraperitoneally (i.p.; Han et al., 2008; Rzeski et al., 2004), or intravenously (i.v.; Helal et al., 2009; Seigers et al., 2010); i.p. administration is more commonly used than i.v. administration. Intraperitoneal injections mimic the slower administration of clinical patients as the drugs slowly diffuse across the abdominal wall and into the blood stream. However, i.p. administration is also more toxic at the site of injection and results in a smaller concentration of drug reaching the blood stream (Aletti et al., 2010). In a clinical study of women with advanced ovarian cancer, women receiving i.p. chemotherapy had more abdominal pain, nausea, vomiting, and other side effects than the women receiving treatment intravenously (Armstrong et al., 2006; Wenzel, Huang, Armstrong, Walker, & Cella, 2007).

Intravenous administration in rodents mimics clinical route of administration. However, bolus injections are given instead of a drip line, resulting in a much larger immediate concentration of chemotherapeutic agents in the blood stream. Some studies have addressed this by administering multiple injections of smaller drug concentrations over a short period of time (Rajamani, Muthuvel, Senthilvelan, & Sheeladevi, 2006). It is not known if these alterations
in drug administration result in different chemotherapy associated changes in
neuropsychological functioning. A few studies have also used
intracerebroventricular or intrathecal injections in order to determine the direct
effect of chemotherapeutic drugs in the brain, bypassing drug metabolism in the
liver and blood brain barrier protection (Lau et al., 2009; Vijayanathan, Gulinello,
Ali, & Cole, 2011). However, this approach is not very relevant to assessing
chemotherapeutic-related effects on cognition and affect as it is very difficult for
most chemotherapy agents to cross the blood brain barrier, this being a major
obstacle when trying to treat CNS tumors (Gerstner & Fine, 2007; Sawyer,
Piepmeier, & Saltzman, 2006).

Another potential roadblock in pre-clinical research is the vast variation in
dosage, frequency and duration of chemotherapeutic treatment. As seen in Table
13, the variation in dosage is small for some drugs such as doxorubicin and
cyclophosphamide, while it is much larger for others such as Methotrexate,
ranging from 0.1 mg/kg to 250 mg/kg for a single treatment. Additionally, rodent
studies vary greatly in the number and frequency of treatments. The studies
shown in Table 13 range from one to ten injections with frequency ranging from
once each day to once each week and duration from first treatment to analysis
ranging from 3 hours to eight weeks (Y. Li, V. Vijayanathan, M. E. Gulinello, & P.
D. Cole, 2010; Tangpong et al., 2006). These differences may result in varying
results, as both dose and number of chemotherapy treatments are associated
with worse neuropsychological outcome (Hodgson, Hutchinson, Wilson, &
Nettelbeck, 2013; Schagen, Muller, Boogerd, Mellenbergh, & van Dam, 2006). In determining a proper dose, it is recommended to calculate the human-equivalent-dose (HED) which is based on surface area instead of height and eight (Reagan-Shaw, Nihal, & Ahmad, 2008). Using similar dosages and frequency and duration of treatments will be beneficial in comparing across future studies as the cellular and molecular mechanisms being tested become more specific and complex.

In the past decade, rodent models have increasingly been used to elucidate the mechanism by which chemotherapeutic agents are associated with neuropsychological alterations. Animal models are able to replicate the chemotherapy-associated deficits seen in clinical studies. However, as research in this area becomes more mechanistic, focusing on cellular and molecular pathways, it will be essential to understand how the differences in species, sex, tumor presence and chemotherapeutic agents might affect cellular, molecular, and immunological pathways differently and to design the studies with these in mind.

As discussed in a review on chemotherapy and animal models (Seigers, Schagen, Van Tellingen, & Dietrich, 2013), future research would benefit greatly from having a shared database in which methods and study designs for all related studies were entered in and readily accessed by everyone. It would also be beneficial for researchers to be able to access negative findings in addition to positive outcomes. For example, researchers might have tried multiple dosages
and frequencies with a specific drug in a specific species before finding one that worked. Having access to this information would facilitate future research.

An important future direction for the preclinical work will be to understand individual variability in susceptibility to the cognitive and affective side-effects of chemotherapy. For some patients, these deficits never emerge, while for others they emerge and are resolved within a year, while for others still, they will persist for decades. A number of psycho-social factors have been postulated as contributing to individual differences in susceptibility to these side effects of treatment, including pre-treatment cognitive ability, socioeconomic status, education, age, and social support (Ahles et al., 2002; Avis et al., 2012; Caplette-Gingras & Savard, 2008; Cimprich, So, Ronis, & Trask, 2005; S. R. Kesler et al., 2013; Kissane et al., 2004), but the relative importance of each of these variables in cognitive and affective deficits remains to be determined. In addition, it has recently been suggested that genetic variations may also be involved; indeed, single nucleotide polymorphisms in promoter regions of cytokine DNA are associated with increased neuropsychological deficits (Bower et al., 2013).

8. Conclusions

Chemotherapy treatment is frequently administered to breast cancer patients to prevent tumor recurrence and has been beneficial in increasing the overall 10-year survival rate, now at 84.5% (Howlader N, 2011). However,
chemotherapeutic agents are also associated with neuropsychological impairment and can have a negative impact on quality of life. Although there are over 1,000 publications examining these side effects, the mechanism by which chemotherapeutic agents are associated with neuropsychological deficits has not been elucidated. This is likely due to the wide variations in study design, cognitive tests, chemotherapy drugs, treatment duration and method of data analysis. However, imaging studies have left little doubt that patients treated with chemotherapy have reduced volume of both white and gray matter, and altered brain activity during cognitive tasks. Furthermore, imaging studies have been more consistent in documenting cognitive deficits than those not utilizing imaging. Future research needs to assess the sensitivity of the tests currently used for cognitive assessment in chemotherapy treated patients.

In the past decade, a growing number of pre-clinical studies using non-tumor bearing rodent models have corroborated clinical findings, reporting that rodents treated with chemotherapy have cognitive deficits and show increased depressive-like and anxiety-like behavior. While it is difficult to assess the effect of each individual drug in clinical research, rodent models have found that many different chemotherapy agents are independently associated with neuropsychological deficits. Furthermore, these studies have proposed potential mechanisms associated with chemotherapeutic neurotoxicity within the CNS involving neurogenesis, cell death, oxidative stress, inflammation,
neurotransmitters and monoamines. However, no interventional studies have attempted to establish causation.

Until the past decade, a majority of clinical studies were descriptive, assessing correlative associations between chemotherapy and cognitive deficits. Accordingly, in 2011 the International Cognition and Cancer Task Force reiterated the importance of appropriate study design and recommended longitudinal studies, focusing on causation. Similarly, still in its infancy, preclinical studies have determined a correlation between chemotherapy and neuropsychological deficits, but have not focused on designing causational studies with an emphasis on translational outcome. Now that a number of possible mechanisms have been suggested using single chemotherapeutic agents, it will be important for future study designs to include the age, sex, hormone status, and drug combination, administration and dosage that will best mimic the clinical setting, with a focus on translational outcomes. Furthermore, a shared database containing detailed information about study design, methods, and results, both positive and negative, would be extremely valuable in facilitating progress in this field. Following these guidelines will assist in translating pre-clinical findings to clinical studies by reducing chemotherapy-associated neuropsychological deficits and increasing quality of life of cancer patients, which will likely reduce the recurrence rate and mortality rate of breast cancer.
<table>
<thead>
<tr>
<th>Instrument</th>
<th>Design</th>
<th>n per group</th>
<th>Controls</th>
<th>Chemo</th>
<th>Conclusion</th>
<th>Notes/Limitations</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCFQ</td>
<td>Longitudinal baseline, 6 mo</td>
<td>43-50</td>
<td>healthy</td>
<td>FEC, CMF, AC</td>
<td>Higher self report deficits for chemotherapy patients than controls, but not correlated with objective scores</td>
<td>Preliminary data from ongoing study with later time point</td>
<td>Schilling 2005</td>
</tr>
<tr>
<td>MASRQ</td>
<td>Longitudinal baseline, 1 mo, 6 mo, 18 mo</td>
<td>45-60</td>
<td>BC-no chemo, healthy</td>
<td>ACP, AC, CAF, FEC, CMF</td>
<td>The increase in cognitive symptoms was greater for chemotherapy patients than patients not treated with chemotherapy and healthy controls</td>
<td>80% of chemo and 66% of BC-no chemo groups on ET. Sample: primarily well-educated Caucasian women.</td>
<td>Ahles 2010</td>
</tr>
<tr>
<td>PAOF</td>
<td>Longitudinal baseline, after chemo, 6 mo later</td>
<td>30-31</td>
<td>BC-no chemo,</td>
<td>ACT, CMF, AC</td>
<td>Self report of memory problems did not differ by treatment; however, they were associated with depression or anxiety scores at various time points.</td>
<td>Older women (~61 yo). Women who withdrew mid-study were more cognitively impaired than the group mean on several objective measures. ET use varied across time points and by group.</td>
<td>Tager 2010</td>
</tr>
<tr>
<td>AFI</td>
<td>Longitudinal baseline, ~1-4 weeks after final chemo infusion</td>
<td>30</td>
<td>none</td>
<td>AC</td>
<td>Women report a significant reduction in perceived cognitive performance from baseline to post-treatment testing.</td>
<td>None of women taking ET at time of testing.</td>
<td>Jansen 2008</td>
</tr>
<tr>
<td>FSCL</td>
<td>Longitudinal cycle 2, cycle 4</td>
<td>27</td>
<td>none</td>
<td>AC/CAF, CMF</td>
<td>Proportion of patients reporting cognitive deficits higher for those receiving AC/CAF versus CMF during chemotherapy or immediately after conclusion.</td>
<td>Among patients receiving AC/CAF changes in IL-6 were positively correlated with all 5 cognitive measures, although not approaching significance in any measure except difficulty thinking (p=0.059).</td>
<td>Janelsins 2012</td>
</tr>
<tr>
<td>Semi-Structured Interview</td>
<td>Cross-sectional ~2 yr after start of treatment</td>
<td>19-34</td>
<td>BC-no chemo</td>
<td>CMF +/- ET</td>
<td>Women treated with chemotherapy (CMF) had significantly lower scores relative to patients who did not receive chemotherapy after surgery</td>
<td>The cognitive score was highly correlated with the emotional functioning scale EORTC-QLQ. Dutch cohort.</td>
<td>Schagen 1999</td>
</tr>
</tbody>
</table>

Continued
<table>
<thead>
<tr>
<th>Instrument</th>
<th>Design</th>
<th>n per group</th>
<th>Controls</th>
<th>Chemo</th>
<th>Conclusion</th>
<th>Notes/Limitations</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMSRQ</td>
<td>Cross-sectional</td>
<td>22-35</td>
<td>BC-no chemo, lymphoma</td>
<td>20 diff. regimens, most containing AC</td>
<td>The chemotherapy group reported greater deficits in working memory than the no chemotherapy group. There was a trend toward impaired new learning (p=0.07) but no significant effect on remote retrieval.</td>
<td>No significant correlation between the SMSRQ subscales and domain scores.</td>
<td>Ahles 2002</td>
</tr>
<tr>
<td>FEDA</td>
<td>Cross-sectional</td>
<td>23-29</td>
<td>BC-no chemo</td>
<td>EC, CMF, CTM</td>
<td>No differences in self-perceived cognitive deficit scores among standard dose chemotherapy, high dose chemotherapy, and non-chemotherapy BC controls. Self perceived cognitive function does not correlate with objective assessments of function.</td>
<td>Two chemotherapy groups more likely to have had mastectomy and higher tumor grade than non-chemotherapy controls. High-risk German cohort using German versions of instruments (some modified).</td>
<td>Mehnert 2007</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Instruments</th>
<th>Design</th>
<th>n per group</th>
<th>Controls</th>
<th>Chemo</th>
<th>Conclusion</th>
<th>Notes/Limitations</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBANS</td>
<td>Longitudinal baseline, ~ 1-4 weeks after final chemo infusion</td>
<td>30</td>
<td>none</td>
<td>AC</td>
<td>From baseline to post-chemo, reduction in total cognitive score and visuospatial skill; no diff. in immediate memory, language, attention, or delayed memory.</td>
<td>None of women taking ET at time of testing.</td>
<td>Jansen 2008</td>
</tr>
<tr>
<td>CNS-VS, WAIS-III, PASAT, CCC, COWA, HLVT-R, BVMT-R</td>
<td>Longitudinal baseline and before each infusion</td>
<td>60</td>
<td>healthy</td>
<td>FEC-T, FEC, CT, AC-T, AC</td>
<td>Cognitive function is impaired at several time points after chemotherapy. Working memory and processing speed appear to be most sensitive to chemotherapy effects.</td>
<td>Progressive worsening of cognitive function during chemotherapy treatment rel. to controls. One year results not yet available. Canadian cohort.</td>
<td>Collins 2013</td>
</tr>
<tr>
<td>PASAT; TMT A and B, WCST; BNT; COWA; GP; DSC; WAIS-III; CVLT II; WMS-III; RVT; CCC</td>
<td>Longitudinal baseline, 1 mo after chemo, 1 year after chemo</td>
<td>40-53</td>
<td>BC-no chemo</td>
<td>FEC (~50%), CEF, CAF, AC, ACT, ECT</td>
<td>Higher rate of decline from baseline to 1 month in the chemo group (34% v 13%); chemo impaired working and visual memory at 2nd time point; exec. function was improved one year later in chemo group. Trend (p=0.06) for lower overall summary in chemo patients receiving ET; sig. deficits were apparent in verbal memory and processing speed.</td>
<td>All participants post-menopausal. Higher tumor grade in chemo group. Canadian cohort. Women who withdrew mid-study were below the mean on several cognitive measures at baseline; at baseline, chemo groups scored higher on several measures.</td>
<td>Collins 2009</td>
</tr>
<tr>
<td>Composite Score from 10 tests (details absent)</td>
<td>Longitudinal baseline, 6 mo after chemo</td>
<td>19-34</td>
<td>BC-no chemo, healthy controls</td>
<td>CTC (high dose), FEC</td>
<td>Greater cog. decline across time in CTC patients</td>
<td>High-risk Dutch cohort.</td>
<td>Schagen 2006</td>
</tr>
<tr>
<td>WAIS-III, WRAT, BNT, COWA, CVLT, WMS-R; CPT</td>
<td>Cross-sectional ~10 years after treatment</td>
<td>22-35</td>
<td>BC-no chemo, lymphoma</td>
<td>20 diff. regimens</td>
<td>Chemo impaired neuropsych. performance; verbal memory and psychomotor functions were most affected.</td>
<td>Correlation between the # of chemotherapy cycles and domain scores. No corr. between cognitive function and time since treatment. No differences in dep. or anx. among groups.</td>
<td>Ahles 2002</td>
</tr>
</tbody>
</table>
### Table 2 continued

<table>
<thead>
<tr>
<th>Instruments</th>
<th>Design</th>
<th>n per group</th>
<th>Controls</th>
<th>Chemo</th>
<th>Conclusion</th>
<th>Notes/Limitations</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMT, TAP, WMS-R, AVLT, ROCFT, RWT, WAIS-R</td>
<td>Cross-sectional ~5 years after treatment</td>
<td>23-29</td>
<td>BC-no chemo</td>
<td>CMF, CMT</td>
<td>Global cognitive impairment in 8% of high-dose, 13% of standard-dose, 3% of non-chemo; no significant difference among groups.</td>
<td>Chemotherapy groups more likely to have had mastectomy and higher tumor grade. High dose also received autologous bone marrow stem cells. High-risk German cohort using German versions of instruments (some modified).</td>
<td>Scherwat h 2006</td>
</tr>
<tr>
<td>FMMSE</td>
<td>Cross-sectional; &gt;10 y post-treatment</td>
<td>30</td>
<td>healthy</td>
<td>CMF, AC</td>
<td>Performance on this task was significantly impaired for BC patients relative to age-matched non-cancer controls</td>
<td>Long term survivors over the age of 65.</td>
<td>Yamada 2010</td>
</tr>
</tbody>
</table>

Table 3. Motor Function

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Design</th>
<th>n per group</th>
<th>Controls</th>
<th>Chemo</th>
<th>Conclusion</th>
<th>Notes/Limitations</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grooved Pegboard</td>
<td>Longitudinal baseline, ~1-4 weeks after final chemo infusion</td>
<td>30</td>
<td>none</td>
<td>AC</td>
<td>There were no significant differences in performance of the dominant or non-dominant (although p=0.06) hand between baseline and after completion of chemotherapy.</td>
<td>None of women taking ET at time of testing.</td>
<td>Jansen 2008</td>
</tr>
<tr>
<td>Grooved Pegboard</td>
<td>Longitudinal baseline, 3 w after chemo, 1 y later.</td>
<td>18</td>
<td>None</td>
<td>FAC</td>
<td>Decline in 1/18 patients relative to baseline at 3 weeks post-chemo</td>
<td></td>
<td>Wefel 2004</td>
</tr>
<tr>
<td>Grooved Pegboard</td>
<td>Cross-sectional within 18 months following chemotherapy</td>
<td>28</td>
<td>None; used normative values</td>
<td>CMF (majority), CMF + CAF</td>
<td>Chemotherapy is associated with a significant decline from normative values in both dominant (~1 SD) and non-dominant (&lt;1 SD) hands.</td>
<td>Younger women (mean: 42 yo).</td>
<td>Wienike 1995</td>
</tr>
<tr>
<td>FePsy Automated Testing (finger tapping)</td>
<td>Cross-sectional ~2 yr after start of treatment</td>
<td>19-34</td>
<td>BC-no chemo</td>
<td>CMF +/- ET</td>
<td>Women treated with chemotherapy had impaired performance on the Finger Tapping task (both dominant and non-dominant) relative to patients who did not receive chemotherapy after surgery</td>
<td>Dutch program and cohort.</td>
<td>Schagen 1999</td>
</tr>
<tr>
<td>Composite Score (Grooved Pegboard, Finger Tapping)</td>
<td>Longitudinal baseline, after chemo, 6 mo later</td>
<td>30-31</td>
<td>BC-no chemo</td>
<td>ACT (majority), AC, CMF</td>
<td>Patients not treated with chemotherapy showed improvement in performance over time whereas those treated with chemotherapy showed a nonsignificant decline from baseline to post-treatment testing approximately 18 months later (p=0.08).</td>
<td>Older post-menopausal women (mean age 61). Women who withdrew mid-study were more impaired than the group mean on several objective measures. Diagnosis, ET use varied by group. ET use also varied by time point.</td>
<td>Tager 2010</td>
</tr>
</tbody>
</table>

AC: adriamyacin, cyclophosphamide; CAF: adriamyacin, cyclophosphamide, 5-fluorouracil; CMF: cyclophosphamide, methotrexate, fluorouracil; ACT: adriamyacin, cyclophosphamide, and a taxane; FAC: fluorouracil, adriamyacin, cyclophosphamide; ET: endocrine therapy.
<table>
<thead>
<tr>
<th>Instrument</th>
<th>Design</th>
<th>n per group</th>
<th>Controls</th>
<th>Chemo</th>
<th>Conclusion</th>
<th>Notes/Limitations</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Letter Cancellation</td>
<td>Longitudinal baseline, 6 mo</td>
<td>43-50</td>
<td>healthy</td>
<td>FEC, CMF, AC</td>
<td>Performance impaired in BC relative to healthy controls; performance improving in both groups with repeated testing.</td>
<td>Composite score based on speed and accuracy.</td>
<td>Shilling 2005</td>
</tr>
<tr>
<td>Letter Cancellation</td>
<td>Longitudinal baseline, 6 mo, 18 mo</td>
<td>43-85</td>
<td>BC-no chemo, healthy</td>
<td>FEC, CMF, AC, EC, ECP, ECMF, EFEC</td>
<td>Cognitive deficits only in a few women.</td>
<td>59% of chemotherapy group were node positive versus 14% of non-chemotherapy.</td>
<td>Jenkins 2006</td>
</tr>
<tr>
<td>TMT-A</td>
<td>Longitudinal baseline, 3 w after chemo, 1 y later</td>
<td>18</td>
<td>None</td>
<td>FAC</td>
<td>Decline in 2/18 patients relative to baseline at 3 weeks post-chemo;</td>
<td></td>
<td>Wefel 2004</td>
</tr>
<tr>
<td>TMT-A</td>
<td>Longitudinal baseline and before each treatment</td>
<td>60</td>
<td>healthy</td>
<td>FEC-T, FEC, CT, AC-T, AC</td>
<td>Chemotherapy impaired performance relative to controls at 2 of the 7 time points spanning treatment.</td>
<td>Canadian cohort.</td>
<td>Collins 2013</td>
</tr>
<tr>
<td>TMT-A</td>
<td>Longitudinal baseline, 6 mo after chemo</td>
<td>28</td>
<td>None</td>
<td>CMF (majority), ACT, AC-T-H</td>
<td>7% experienced a 2 SD decline in performance from baseline to post-chemotherapy testing.</td>
<td>Older women (mean: 71 yo). Protocol was modified 25% of the way through data collection.</td>
<td>Hurria 2006</td>
</tr>
<tr>
<td>TMT-A</td>
<td>Longitudinal baseline, 6 mo after chemo</td>
<td>101</td>
<td>None</td>
<td>EC+P or E+P+CMF</td>
<td>Similar to norms at both time points</td>
<td>Preoperative chemotherapy. German cohort and version of instruments. Imperfectly matched normative samples were used to estimate practice effects.</td>
<td>Hermelink 2007</td>
</tr>
<tr>
<td>TMT-A</td>
<td>Longitudinal baseline, immediately post-chemo, 3 mo later</td>
<td>40-45</td>
<td>BC-no chemo, healthy</td>
<td>AC, TAC, FEC</td>
<td>Performance improved over time from baseline to 3 months post-treatment in both chemotherapy and radiotherapy patients.</td>
<td>Canadian cohort Diff. in age, tumor stage between cancer groups. Control group was tested only once, so practice effects could not be determined in this group.</td>
<td>Quesnel 2009</td>
</tr>
</tbody>
</table>
### Table 4 continued

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Design</th>
<th>n per group</th>
<th>Controls</th>
<th>Chemo</th>
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<tbody>
<tr>
<td>TMT-A</td>
<td>Longitudinal baseline, 1-2 mo post-chemo</td>
<td>12-34</td>
<td>Cardiac patients, healthy controls</td>
<td>CEF</td>
<td>Performance improved with repeated testing. No group differences.</td>
<td>Group differences in age, sex, and education. Danish cohort.</td>
<td>Mehlsen 2008</td>
</tr>
<tr>
<td>TMT-A</td>
<td>Cross-sectional Within 18 months following chemotherapy</td>
<td>28</td>
<td>None; used normative values</td>
<td>CMF (majority), CMF + CAF, CAF</td>
<td>A significant decline in women tested within 18 months of completing chemotherapy relative to normative values; but they remain within 1 SD below normal.</td>
<td>Younger women (mean: 42 yo).</td>
<td>Weinke 1995</td>
</tr>
<tr>
<td>TMT-A</td>
<td>Cross-sectional; &gt;10 y post-treatment</td>
<td>30</td>
<td>healthy</td>
<td>CMF, AC</td>
<td>Performance was significantly impaired in BC survivors relative to healthy controls.</td>
<td>Long term survivors over the age of 65.</td>
<td>Yamada 2010</td>
</tr>
<tr>
<td>TMT-A</td>
<td>Cross-sectional ~2 yr after start of treatment</td>
<td>19-34</td>
<td>BC-no chemo</td>
<td>CMF +/- ET</td>
<td>Women treated with chemotherapy exhibited a non-significant trend toward impaired performance relative to patients who did not receive chemotherapy after surgery (p=0.08).</td>
<td>Dutch cohort.</td>
<td>Schagen 1999</td>
</tr>
<tr>
<td>Symbol Digit Modalities Test</td>
<td>Longitudinal baseline, immediately post-chemo, 3 mo later</td>
<td>40-45</td>
<td>BC-no chemo, healthy</td>
<td>AC, AC-T, FEC</td>
<td>Radiation patients had lower written results than healthy controls. A non-significant trend in the same direction existed for the oral portion; no apparent chemotherapy effect.</td>
<td>Canadian cohort Diff. in age, tumor stage between cancer groups. Control group was tested only once, so practice effects could not be determined in this group.</td>
<td>Quesnel 2009</td>
</tr>
<tr>
<td>Digit Symbol Coding (WAIS-R)</td>
<td>Longitudinal baseline, 3 w after chemo, 1 y later.</td>
<td>18</td>
<td>None</td>
<td>FAC</td>
<td>Decline in 2/18 patients relative to baseline at 3 weeks post-chemo.</td>
<td>Dutch cohort.</td>
<td>Wefel 2004</td>
</tr>
<tr>
<td>Digit Symbol Coding (WAIS-R)</td>
<td>Cross-sectional Within 18 months following chemotherapy</td>
<td>28</td>
<td>None; used normative values</td>
<td>CMF (majority), CMF + CAF, CAF</td>
<td>No significant deviation from normative values among women tested within 18 months of completing chemotherapy</td>
<td>Younger women (mean: 42 yo).</td>
<td>Wienke 1995</td>
</tr>
</tbody>
</table>

Continued
### Abbreviations:
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<tbody>
<tr>
<td>RCF-IR, RCF-DR, RCF-C</td>
<td>Longitudinal baseline, 6 mo, 18 mo</td>
<td>43-85</td>
<td>BC-no chemo, healthy</td>
<td>FEC, CMF, AC, EC, ECP, ECMF, EFEC</td>
<td>Cognitive deficits only in a few women.</td>
<td>59% of chemotherapy group were node positive versus 14% of non-chemotherapy.</td>
<td>Jenkins 2006</td>
</tr>
<tr>
<td>RCF-IR, RCF-DR, RCF-C</td>
<td>Longitudinal baseline, 6 mo</td>
<td>43-50</td>
<td>healthy</td>
<td>FEC, CMF, AC</td>
<td>Chemo therapy had no effect.</td>
<td>Composite score based on speed and accuracy.</td>
<td>Shilling 2005</td>
</tr>
<tr>
<td>RCF-IR, RCF-DR, RCF-C</td>
<td>Longitudinal baseline, 1 wk after completed chemo, 1 yr later</td>
<td>12-19</td>
<td>BC-no chemo,</td>
<td>AC +/- ET (majority) CMF +/- ET</td>
<td>AC+ET group declined in performance on both measures from T2 to T3 and was significantly lower than AC+noET at T3 for RCF-IR</td>
<td>Very small n and high attrition.</td>
<td>Bender 2006</td>
</tr>
<tr>
<td>RCF-C</td>
<td>Longitudinal baseline, after chemo, 6 mo later</td>
<td>30-31</td>
<td>BC-no chemo</td>
<td>ACT (majority), AC, CMF</td>
<td>There was improved performance across testing at baseline, post treatment and 6 months post-treatment, but no chemo effect</td>
<td>Older post-menopausal women (mean age 61). Diagnosis and ET use varied by group. ET use also varied by time point.</td>
<td>Tager 2010</td>
</tr>
<tr>
<td>RCF-IR, RCF-DR, RCF-C</td>
<td>Longitudinal baseline, immediately post-chemo, 3 mo later</td>
<td>40-45</td>
<td>BC-no chemo, healthy</td>
<td>AC, AC-T, FEC</td>
<td>Performance on the copy immediate and delayed recalls improved over time from baseline to 3 months post-treatment in both chemotherapy and radiotherapy patients</td>
<td>Canadian cohort. Diff. in age, tumor stage between cancer groups. Control group tested only once, so practice effects could not be determined.</td>
<td>Quesnel 2009</td>
</tr>
<tr>
<td>RCF-IR, RCF-DR, RCF-C</td>
<td>Longitudinal baseline, 6 mo after chemo</td>
<td>28</td>
<td>None</td>
<td>CMF (majority), ACT, AC, AC-T-H</td>
<td>Decline in RCFT Copy, Immediate Recall, and Delayed Recall from baseline to 6 months after chemotherapy.</td>
<td>Older women (mean age 71). Protocol was modified 25% of the way through data collection to reduce participant burden.</td>
<td>Hurria 2006</td>
</tr>
<tr>
<td>RCF-IR, RCF-DR, RCF-C</td>
<td>Longitudinal baseline, 1-2 mo post-chemo</td>
<td>12-34</td>
<td>Cardiac patients, healthy controls</td>
<td>CEF</td>
<td>No sig. change from pre-chemo to 2 months post-treatment; no differences relative to healthy controls or cardiac patients</td>
<td>Group differences in age, sex, and education. Danish cohort.</td>
<td>Mehlisen 2008</td>
</tr>
<tr>
<td>RCF-DR, RCF-C</td>
<td>Cross-sectional Within 18 months following chemotherapy</td>
<td>28</td>
<td>None; used normative values</td>
<td>CMF (majority), CMF + CAF, CAF</td>
<td>Significant declines of greater than 1SD from normative values among women tested within 18 months of completing chemotherapy</td>
<td>Younger women (mean: 42 yo).</td>
<td>Wiencke 1995</td>
</tr>
<tr>
<td>RCF-IR, RCF-DR, RCF-C</td>
<td>Cross-sectional ~2 yr after start of treatment</td>
<td>19-34</td>
<td>BC-no chemo</td>
<td>CMF +/- ET</td>
<td>Chemo patients had significantly lower scores.</td>
<td>Dutch cohort.</td>
<td>Schagen 1999</td>
</tr>
</tbody>
</table>

Continued
The performance of BC patients was similar to age-matched non-cancer controls. Long term survivors over the age of 65. Yamada 2010

No significant difference between healthy controls and chemotherapy patients at any of the 7 time points spanning treatment. Canadian cohort. Collins 2013

No sig. difference although it approached significance (p=0.08; lower in chemo group). Long term survivors over the age of 65. Yamada 2010

Decline in 1/18 patients relative to baseline at 3 weeks post-chemo. All participants post-menopausal. Higher tumor grade in chemo group. Canadian cohort. At baseline, chemo groups scored higher on several measures. Collins 2009

At baseline, the chemo group had impaired performance relative to the non-chemo group. Older post-menopausal women (mean age 61). Diagnosis and ET use varied by group, time point. Tager 2010

Abbreviations: BVRT: Benton Visual Retention Test –Revised; BFRT: Benton Facial Recognition Test; BSRT: Buschke Selective Reading Test; BVMT: Brief Visuospatial Memory Test; RCF-IR: Rey Complex Figure Immediate Recall, RCF-TD: Rey Complex Figure Task Delay; RCF-C Rey Complex Figure Copy; RCF-R: Rey Complex Figure Recognition; VSRT: visuo-spatial reasoning task; RCF-DR: Rey Complex Figure Delayed Recall; VSRT: Verbal Selective Reminding Test; NVSRT: Nonverbal Selective Reminding Test.

Table 6. Working Memory and Executive Function

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Design</th>
<th>n per group</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Stroop</td>
<td>Longitudinal baseline, 1-4 weeks after last chemo</td>
<td>30</td>
<td>none</td>
<td>AC</td>
<td>Women treated with chemotherapy showed improved performance on this task from baseline to post-treatment testing.</td>
<td>None of women taking ET at time of testing.</td>
<td>Jansen 2008</td>
</tr>
<tr>
<td>Stroop</td>
<td>Longitudinal baseline, 6 mo</td>
<td>43-50</td>
<td>healthy</td>
<td>FEC, CMF, AC</td>
<td>No group differences, no change over time.</td>
<td>Composite score based on speed and accuracy.</td>
<td>aShilling 2005</td>
</tr>
<tr>
<td>Stroop</td>
<td>Longitudinal baseline, 6 mo, 18 mo</td>
<td>43-85</td>
<td>BC-no chemo, healthy</td>
<td>FEC, CMF, AC, EC, ECP, ECMF, EFEC</td>
<td>No group differences, improved performance over time.</td>
<td>59% of chemotherapy group were node positive versus 14% of non-chemotherapy.</td>
<td>Jenkins 2006</td>
</tr>
<tr>
<td>DS</td>
<td>Cross-sectional</td>
<td>Within 18 months post chemo</td>
<td>28</td>
<td>None; used normative values</td>
<td>CMF (majority), CMF + CAF, CAF</td>
<td>Chemo group had lower performance relative to normative values, but were still within 1 SD below normal</td>
<td>Younger women (mean: 42 yo).</td>
</tr>
<tr>
<td>DS</td>
<td>Longitudinal baseline, immediately post-chemo, 3 mo later</td>
<td>40-61</td>
<td>BC-no chemo, healthy</td>
<td>AC, AC-T, FEC</td>
<td>Baseline performance before initiating chemotherapy was reduced relative to healthy controls.</td>
<td>Canadian cohort. Diff. in age, tumor stage between cancer groups. Control group tested once, so practice effect undetermined.</td>
<td>Quesnel 2009</td>
</tr>
<tr>
<td>DS</td>
<td>Longitudinal baseline, immediately post-chemo</td>
<td>51-61</td>
<td>BC-no chemo</td>
<td>FEC(majority), CEF, FAC, AC, ACT, ECT</td>
<td>Freq. of decline was greater in chemo patients than HRT across time.</td>
<td>Canadian cohort. Includes only post-menopausal women.</td>
<td>Stewart 2008</td>
</tr>
<tr>
<td>DS</td>
<td>Longitudinal baseline, 6 mo after chemo</td>
<td>101</td>
<td>None</td>
<td>ECP or EPCMF</td>
<td>Women being treated for breast cancer were similar to test norms at the pre-chemotherapy baseline and better than test norms at the end of chemotherapy.</td>
<td>Preoperative chemotherapy. German cohort and version of instruments. Imperfectly matched normative samples were used to estimate practice effects.</td>
<td>Hermelink 2007</td>
</tr>
<tr>
<td>DS</td>
<td>Cross-sectional; &gt;10 y post-chemo</td>
<td>30</td>
<td>healthy</td>
<td>CMF, AC</td>
<td>Performance of BC patients was similar to age-matched non-cancer controls.</td>
<td>Long term survivors over the age of 65.</td>
<td>Yamada 2010</td>
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<tr>
<th>Instrument</th>
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<th>Notes/Limitations</th>
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<tbody>
<tr>
<td>DS/ DSB</td>
<td>Cross-sectional; ~2 yr after start of treatment</td>
<td>19-34</td>
<td>BC-no chemo</td>
<td>CMF +/- ET</td>
<td>Chemotherapy was associated with significantly lower scores on the DSB; there was no group difference in DS.</td>
<td>Dutch cohort and instrument.</td>
<td>Schagen 1999</td>
</tr>
<tr>
<td>DSB</td>
<td>Longitudinal baseline, 6 mo</td>
<td>43-50</td>
<td>healthy</td>
<td>FEC, CMF, AC</td>
<td>Deficit detectable 6 mo after chemo.</td>
<td>Composite score based on speed and accuracy.</td>
<td>Shilling 2005</td>
</tr>
<tr>
<td>CCC</td>
<td>Longitudinal baseline, immediately post-chemo</td>
<td>51-61</td>
<td>BC-no chemo</td>
<td>FEC(majority), CEF, FAC, AC, ACT, ECT</td>
<td>Freq. of decline was greater in chemo patients than HRT across time.</td>
<td>Canadian cohort. Includes only post-menopausal women.</td>
<td>Stewart 2008</td>
</tr>
<tr>
<td>CCC</td>
<td>Longitudinal baseline and before each chemo treatment</td>
<td>60</td>
<td>healthy</td>
<td>FEC-T (majority), FEC, CT, AC-T, AC.</td>
<td>Significant reduction in performance relative to healthy controls apparent as early following first chemotherapy treatment.</td>
<td>Canadian cohort.</td>
<td>Collins 2013</td>
</tr>
<tr>
<td>WCST</td>
<td>Cross-sectional; &gt;10 y post-chemo</td>
<td>30</td>
<td>healthy</td>
<td>CMF, AC</td>
<td>BC patients committed significantly more perseverative errors than age-matched non-cancer controls.</td>
<td>Long term survivors over the age of 65.</td>
<td>Yamada 2010</td>
</tr>
<tr>
<td>WMS-LNS</td>
<td>Longitudinal baseline, 6 mo</td>
<td>43-50</td>
<td>healthy</td>
<td>FEC, CMF, AC</td>
<td>There was a significant time*group interaction; controls improved while BC patients did not.</td>
<td>Composite score based on speed and accuracy.</td>
<td>Shilling 2005</td>
</tr>
<tr>
<td>WMS-LNS</td>
<td>Longitudinal baseline, 1-2 mo post-chemo</td>
<td>12-34</td>
<td>Cardiac patients, healthy controls</td>
<td>CEF</td>
<td>No group differences, no change over time.</td>
<td>Group differences in age, sex, and education. Danish cohort.</td>
<td>Mehlsen 2008</td>
</tr>
<tr>
<td>WMS-LNS</td>
<td>Longitudinal baseline and before each chemo treatment</td>
<td>60</td>
<td>healthy</td>
<td>FEC-T (majority), FEC, CT, AC-T, AC.</td>
<td>No significant difference between healthy controls and chemotherapy patients at any of the 7 time points spanning treatment</td>
<td>Canadian cohort.</td>
<td>Collins 2013</td>
</tr>
<tr>
<td>WMS-LNS</td>
<td>Cross-sectional; &gt;10 y post-chemo</td>
<td>30</td>
<td>healthy</td>
<td>CMF, AC</td>
<td>BC patients were significantly impaired relative to age-matched non-cancer controls.</td>
<td>Long term survivors over the age of 65. No similar difference for Digit Span or Arithmetic Score.</td>
<td>Yamada 2010</td>
</tr>
</tbody>
</table>
Table 6 continued

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<tr>
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<th>Chemo</th>
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<th>Notes/Limitations</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMT-B</td>
<td>Longitudinal baseline, 6 mo after chemo</td>
<td>101</td>
<td>None</td>
<td>ECP or EPCMF</td>
<td>Chemotherapy was associated with impaired performance on this task relative to test norms at the pre-chemotherapy baseline time point; there were no post-chemotherapy group differences.</td>
<td>Preoperative chemotherapy. German cohort and version of instruments. Imperfectly matched normative samples were used to estimate practice effects.</td>
<td>Hermelink 2007</td>
</tr>
<tr>
<td>TMT-B</td>
<td>Cross-sectional; &gt;10 y post-chemo</td>
<td>30</td>
<td>healthy</td>
<td>CMF, AC</td>
<td>Performance on this task was significantly impaired for BC patients relative to age-matched non-cancer controls.</td>
<td>Long term survivors over the age of 65.</td>
<td>Yamada 2010</td>
</tr>
<tr>
<td>TMT-B</td>
<td>Cross-sectional ~2 yr after start of treatment</td>
<td>19-34</td>
<td>BC-no chemo</td>
<td>CMF +/- ET</td>
<td>Women treated with chemotherapy exhibited a significant impairment relative to patients who did not receive chemotherapy after surgery.</td>
<td>Dutch cohort and instrument.</td>
<td>Schagen 1999</td>
</tr>
<tr>
<td>TMT-B</td>
<td>Longitudinal baseline and before each chemo treatment</td>
<td>60</td>
<td>healthy</td>
<td>FEC-T (majority), FEC, CT, AC-T, AC.</td>
<td>No significant difference between healthy controls and chemotherapy patients at any of the 7 time points spanning treatment.</td>
<td>Canadian cohort.</td>
<td>Collins 2013</td>
</tr>
<tr>
<td>TMT-B</td>
<td>Cross-sectional Within 18 months post chemo</td>
<td>28</td>
<td>None; used normative values</td>
<td>CMF (majority), CMF + CAF, CAF</td>
<td>No significant deviation from normative values among women tested within 18 months of completing chemotherapy.</td>
<td>Younger women (mean: 42 yo).</td>
<td>Wiencke 1995</td>
</tr>
<tr>
<td>TMT-B</td>
<td>Longitudinal baseline, immediately post-chemo, 3 mo later</td>
<td>40-45</td>
<td>BC-no chemo, healthy</td>
<td>AC, AC-T, FEC</td>
<td>Performance on the copy immediate and delayed recalls improved over time from baseline to 3 months post-treatment in both groups.</td>
<td>Canadian cohort. Diff. in age, tumor stage between cancer groups. Control group tested once; so practice effect undetermined.</td>
<td>Quesnel 2009</td>
</tr>
<tr>
<td>I/E Shift20 task</td>
<td>Cross-sectional; &gt;10 y post-chemo</td>
<td>30</td>
<td>healthy</td>
<td>CMF, AC</td>
<td>Performance significantly impaired in BC patients controls.</td>
<td>Long term survivors over the age of 65.</td>
<td>Yamada 2010</td>
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Continued
Table 6 continued

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</thead>
<tbody>
<tr>
<td>Composite (WAIS-III tasks and WMS-III SS)</td>
<td>Longitudinal baseline, 1 mo after chemo, 1 year after chemo</td>
<td>40-53</td>
<td>BC-no chemo</td>
<td>FEC (~50%), CEF, CAF, AC, ACT, ECT</td>
<td>Summary score decline between first two times was greater in chemo group; no significant differences 1 year later.</td>
<td>All participants post-menopausal. Higher tumor grade in chemo group. Canadian cohort. At baseline, chemo groups scored higher on several measures.</td>
<td>Collins 2009</td>
</tr>
<tr>
<td>Working Memory &amp; Attention Composite (TMT, DS,NLS, Arithmetic)</td>
<td>Longitudinal baseline, after chemo, 6 mo later</td>
<td>30-31</td>
<td>BC-no chemo</td>
<td>ACT (majority), AC, CMF</td>
<td>There was no apparent treatment or time effect at baseline, post treatment or 6 months post-treatment among breast cancer patients who received or did not receive chemotherapy.</td>
<td>Study conducted in older post-menopausal women (mean age 61). Women who withdrew mid-study were more cognitively impaired than the group mean on several objective measures. Diagnosis and ET use varied by group. ET use also varied by time point.</td>
<td>Tager 2010</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Design</th>
<th>n per group</th>
<th>Controls</th>
<th>Chemo</th>
<th>Conclusion</th>
<th>Notes/Limitations</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>HVLT-R</td>
<td>Longitudinal baseline and before each treatment</td>
<td>60</td>
<td>healthy</td>
<td>FEC-T, FEC, CT, AC-T, AC</td>
<td>Difference in total score between groups at 1/7 time points during treatment.</td>
<td>Canadian cohort.</td>
<td>Collins 2013</td>
</tr>
<tr>
<td>AVLT</td>
<td>Longitudinal baseline, 6 mo</td>
<td>43-50</td>
<td>healthy</td>
<td>FECH, CMF, AC</td>
<td>A significant time*group interaction existed for both Supraspan and Total recall: controls improved while BC patients declined.</td>
<td>Composite score based on speed and accuracy.</td>
<td>Shilling 2005</td>
</tr>
<tr>
<td>AVLT</td>
<td>Longitudinal baseline, 6 mo, 18 mo</td>
<td>43-85</td>
<td>BC-no chemo, healthy</td>
<td>FECH, CMF, AC, EC, ECP, ECMF, EFEC</td>
<td>No significant time or group effects.</td>
<td>59% of chemotherapy group were node positive versus 14% of non-chemotherapy.</td>
<td>Jenkins 2006</td>
</tr>
<tr>
<td>AVLT</td>
<td>Cross-sectional ~2 yr after start of treatment</td>
<td>19-34</td>
<td>BC-no chemo</td>
<td>CMF +/- ET</td>
<td>Chemotherapy was associated with an impairment in delayed recall, but not immediate recall or recognition, relative to patients who did not receive chemotherapy after surgery (p=0.08)</td>
<td>Dutch cohort.</td>
<td>Schagen 1999</td>
</tr>
<tr>
<td>AVLT</td>
<td>Cross-sectional; &gt;10 y post-chemo</td>
<td>30</td>
<td>healthy</td>
<td>CMF, AC</td>
<td>BC patients committed significantly more perseverative errors than age-matched non-cancer controls.</td>
<td>Long term survivors over the age of 65.</td>
<td>Yamada 2010</td>
</tr>
<tr>
<td>AVLT</td>
<td>Longitudinal baseline, 1-2 mo post-chemo</td>
<td>12-34</td>
<td>Cardiac patients, healthy controls</td>
<td>CEF</td>
<td>No significant time or group effects.</td>
<td>Group differences in age, sex, and education. Danish cohort.</td>
<td>Mehlsen 2008</td>
</tr>
<tr>
<td>CVLT</td>
<td>Longitudinal baseline, 1 mo after chemo, 1 year after chemo</td>
<td>40-53</td>
<td>BC-no chemo</td>
<td>FEC (~50%), CEF, CAF, AC, ACT, ECT</td>
<td>Baseline performance was significantly better among patients who subsequently received chemotherapy versus those who had hormone therapy without chemotherapy.</td>
<td>All post-menopausal. Higher tumor grade in chemo group. Canadian cohort. At baseline, chemo groups scored higher on several measures.</td>
<td>Collins 2009</td>
</tr>
<tr>
<td>CVLT</td>
<td>Cross-sectional Within 18 months following chemotherapy</td>
<td>28</td>
<td>None; used normative values</td>
<td>CMF (majority), CMF + CAF, CAF</td>
<td>A significant decline from normative values in the Long Delay, but still within 1 SD of normal. No effect on CVLT 1-5 or Short Delay Tests.</td>
<td>Younger women (mean: 42 yo).</td>
<td>Wieneke 1995</td>
</tr>
<tr>
<td>Instrument</td>
<td>Design</td>
<td>n per group</td>
<td>Controls</td>
<td>Chemo</td>
<td>Conclusion</td>
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</tr>
<tr>
<td>CVLT</td>
<td>Cross-sectional; ~10 years after treatment</td>
<td>22-35</td>
<td>BC-no chemo, lymphoma</td>
<td>20 diff. regimens</td>
<td>Scores were comparable for BC and lymphoma patients treated with chemotherapy versus local therapy nearly a decade after treatment.</td>
<td>Ahles 2002</td>
<td></td>
</tr>
<tr>
<td>BNT</td>
<td>Cross-sectional; &gt;10 y post-chemo</td>
<td>30</td>
<td>healthy</td>
<td>CMF, AC</td>
<td>Performance was similar for BC patients and age-matched non-cancer controls.</td>
<td>Long term survivors over the age of 65.</td>
<td>Yamada 2010</td>
</tr>
<tr>
<td>COWAT</td>
<td>Longitudinal baseline and before each treatment</td>
<td>60</td>
<td>healthy</td>
<td>FEC-T, FEC, CT, AC-T, AC</td>
<td>Significant impairment in chemotherapy patients emerge at later time points spanning treatment.</td>
<td>Canadian cohort.</td>
<td>Collins 2013</td>
</tr>
<tr>
<td>Composite: BNT + COWAT</td>
<td>Longitudinal baseline, after chemo, 6 mo later</td>
<td>30-31</td>
<td>BC-no chemo</td>
<td>ACT (majority), AC, CMF</td>
<td>There was improved performance across testing at baseline, post treatment and 6 months post-treatment, but no difference between breast cancer patients who received or did not receive chemotherapy.</td>
<td>Older post-menopausal women (mean age 61). Diagnosis and ET use varied by group. ET use also varied by time point</td>
<td>Tager 2010</td>
</tr>
<tr>
<td>VFT</td>
<td>Longitudinal baseline, immediately post-chemo, 3 mo later</td>
<td>40-45</td>
<td>BC-no chemo, healthy</td>
<td>AC, AC-T, FEC</td>
<td>Performance on the task decreased from baseline to the post-treatment time point among chemotherapy patients but not radiotherapy patients</td>
<td>Canadian cohort. Diff. in age, tumor stage between cancer groups. Control group tested once; so practice effect undetermined.</td>
<td>Quesnel 2009</td>
</tr>
<tr>
<td>4WSMT</td>
<td>Longitudinal baseline, 1 wk after chemo, 1 yr later</td>
<td>12-19</td>
<td>BC-no chemo,</td>
<td>AC+/+ET (majority) CMF+/-ET</td>
<td>Chemo +/-ET did worse on the 5-sec, 15-sec and 30-sec tasks than non-chemo group when tested approximately 1 year after chemo.</td>
<td>Very small n and high attrition.</td>
<td>Bender 2006</td>
</tr>
<tr>
<td>WMS-LM</td>
<td>Longitudinal baseline, 6 mo</td>
<td>43-50</td>
<td>healthy</td>
<td>FEC, CMF, AC</td>
<td>Baseline difference existed between BC and controls prior to chemo.</td>
<td>Composite score based on speed and accuracy.</td>
<td>Shilling 2005</td>
</tr>
<tr>
<td>WMS-LM</td>
<td>Longitudinal baseline, 6 mo, 18 mo</td>
<td>43-85</td>
<td>BC-no chemo, healthy</td>
<td>FEC, CMF, AC, EC, ECMF, EFEC</td>
<td>No significant time or group effects.</td>
<td>59% of chemotherapy group were node positive versus 14% of non-chemotherapy.</td>
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Continued
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<tbody>
<tr>
<td>WMS-LM</td>
<td>Longitudinal baseline, 1 mo after chemo, 1 year after chemo</td>
<td>40-53</td>
<td>BC-no chemo</td>
<td>FEC (~50%), CEF, CAF, AC, ACT, ECT</td>
<td>Baseline performance was significantly better among patients who subsequently received chemotherapy versus those who hormone therapy without chemotherapy.</td>
<td>All post-menopausal. Higher tumor grade in chemo group. Canadian cohort. At baseline, chemo groups scored higher on several measures.</td>
<td>Collins 2009</td>
</tr>
<tr>
<td>WMS-LM</td>
<td>Longitudinal baseline, 1-2 mo post-chemo</td>
<td>12-34</td>
<td>Cardiac patients, healthy controls</td>
<td>CEF</td>
<td>No significant time or group effects.</td>
<td>Group differences in age, sex, and education. Danish cohort.</td>
<td>Mehlsen 2008</td>
</tr>
<tr>
<td>WMR-LM</td>
<td>Cross-sectional ~10 years after treatment</td>
<td>22-35</td>
<td>BC-no chemo, lymphoma</td>
<td>20 diff. regimens</td>
<td>Scores in logical memory I and II were lower for breast cancer and lymphoma patients treated with chemotherapy versus local therapy.</td>
<td></td>
<td>Ahles 2002</td>
</tr>
<tr>
<td>WMR-LM</td>
<td>Longitudinal baseline, 6 mo after chemo</td>
<td>101</td>
<td>None</td>
<td>ECP or EPCMF</td>
<td>Women being treated for breast cancer were similar to test norms at the pre-chemotherapy baseline and better than test norms at the end of chemotherapy.</td>
<td>Preoperative chemotherapy. German cohort and version of instruments. Imperfectly matched normative samples were used to estimate practice effects.</td>
<td>Hermelink 2007</td>
</tr>
<tr>
<td>Composite Score for Verbal Ability (WASI, D-DEFS)</td>
<td>Longitudinal baseline, 1 mo, 6 mo, 18 mo</td>
<td>45-60</td>
<td>BC-no chemo, healthy</td>
<td>ACP, AC, CAF, FEC, CMF</td>
<td>Chemotherapy had an acute effect on verbal ability that was detected upon completion of treatment and then resolved over the subsequent 18 months.</td>
<td>80% of chemo and 66% of BC-no chemo groups on ET. Sample: primarily well-educated Caucasian women.</td>
<td>Ahles 2010</td>
</tr>
</tbody>
</table>

### Table 8. Effects of Chemotherapy on Affect in Breast Cancer Patients (Women)

<table>
<thead>
<tr>
<th>Study Type</th>
<th>Instrument</th>
<th>Drug</th>
<th>Population</th>
<th>Testing Time</th>
<th>Effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Longitudinal</td>
<td>HADS</td>
<td>FEC (6-7 cycles)</td>
<td>n=382 chemo (42%) or non-chemo RT (76.7%), tamoxifen (65.2%); premenopausal (24.9) mean age: 55.62</td>
<td>4,6,10 &amp; 16 months post-treatment</td>
<td>Twice as many chemo patients had high anxiety scores; no difference in depression.</td>
<td>Björneklett 2012</td>
</tr>
<tr>
<td>Longitudinal</td>
<td>IDS-SR PSS</td>
<td>Not stated</td>
<td>n=64 ; 24 chemo, 40 non-chemo mean age: 52, 59 RT: 100%</td>
<td>After chemo, during post-chemo RT and 6 week post RT</td>
<td>Chemo, not radiation, associated w/ depression, IL-6, NF-kB, TNFaR2 and IL-6</td>
<td>Torres 2013</td>
</tr>
<tr>
<td>Longitudinal</td>
<td>HADS</td>
<td>Not stated</td>
<td>n=220 75% HRT; RT: 88% Mean age: 52</td>
<td>Baseline (within 9mo post chemo) 6,12 mo post baseline</td>
<td>No difference in anxiety or depression scores across time</td>
<td>Kwiatkowski 2013</td>
</tr>
<tr>
<td>Longitudinal</td>
<td>PHQ-9 GAD-7</td>
<td>CMF (6),Taxol(15) AC (21)</td>
<td>n=32 mean age 52.4 premenopausal (5.7%)</td>
<td>Weekly over 5 mos; after chemo started (90%)</td>
<td>Dep. associated with AC chemo not others; chemo duration, 1st chemo regimen, anxiety</td>
<td>Reece 2013</td>
</tr>
<tr>
<td>Longitudinal</td>
<td>HADS</td>
<td>FEC, FEC+T, FEA, CAF, CA, CA+T, CA+Taxol, CEA, CMF, C+T</td>
<td>n=138 chemo, 21 non-chemo Mean age: 49, 54</td>
<td>Pre-chemo and 1 month post-chemo</td>
<td>Anxiety, not dep., associated with cognition across time. Cog. assoc. with baseline dep. and fatigue.</td>
<td>Vearncombe 2009</td>
</tr>
<tr>
<td>Cross-sectional</td>
<td>POMS</td>
<td>Not reported</td>
<td>n=175 women, n=88 men Mean age: 57 17.5% BC; many other cancer types also included</td>
<td>While on chemo treatment</td>
<td>Depression, anxiety, fatigue, insomnia all related. Depression is the best predictor of QoL.</td>
<td>Redecker 2000</td>
</tr>
<tr>
<td>Cross-sectional</td>
<td>BDI-1A</td>
<td>Doxorubicin or other</td>
<td>n= 653 women in 4 age groups: 25-44, 45-54, 55-64, 65-74; RT: 25%</td>
<td>Within 8 mo of diagnosis</td>
<td>Dox treatment increase depression, others did not</td>
<td>Avis 2012</td>
</tr>
<tr>
<td>Cross-sectional</td>
<td>BDI</td>
<td>Not listed</td>
<td>n=534; chemo and non-chemo Mean age: 48,49 respectively HRT(%) 69,78; RT(%): 56,51 Premenopausal (%): 33,36</td>
<td>Within 1 year post chemo, 1-3 years, or 3+ years</td>
<td>Higher depression, lower QoL in groups &lt;1year and 3+ yrs since chemo Compared to non-chemo</td>
<td>Hwang 2013</td>
</tr>
<tr>
<td>Study Type</td>
<td>Instrument</td>
<td>Drug</td>
<td>Population</td>
<td>Testing Time</td>
<td>Effect</td>
<td>Reference</td>
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<tr>
<td>Cross-sectional</td>
<td>HADS-A, HADS-D</td>
<td>Not listed</td>
<td>n=72; Metastatic BC</td>
<td>Right before receiving</td>
<td>high IL-6 associated w/ depression, not anxiety. Depression and anx. correlated</td>
<td>Jehn 2012</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>mean age: 60</td>
<td>one of their chemotherapy treatments</td>
<td></td>
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<td></td>
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<td></td>
<td>previous chemo (75%) previous HRT(25%)</td>
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<tr>
<td>Cross-sectional</td>
<td>BDI-II</td>
<td>Not listed</td>
<td>n=103; post-chemo (48%), non-chemo (52%) Mean age: 51</td>
<td>Within 3mo post-treatment</td>
<td>Fatigue, sleep disturbance, depression, and sTNF-rII higher in chemo group</td>
<td>Bower 2011</td>
</tr>
<tr>
<td>Cross-sectional</td>
<td>POMS-SF</td>
<td>AC (6)</td>
<td>n=46 Mean age: 53.38</td>
<td>1mo post chemo</td>
<td>Depression, but not cognition predicted poor QoL</td>
<td>Reid-Arndt 2009</td>
</tr>
<tr>
<td>Cross-sectional</td>
<td>BDI-II FACT-B</td>
<td>30% CMF, 23% AC, 20.5% AT, 26.5% other</td>
<td>n=200 mean age :45 years</td>
<td>While on chemo</td>
<td>Treatment duration (months) and # of chemo cycles associated with menopausal symptoms and sexual dysfunction, but not depression</td>
<td>Park 2013</td>
</tr>
<tr>
<td>Cross-sectional</td>
<td>HADS</td>
<td>Not listed</td>
<td>n=218 chemo (60%) or RT (40%) Mean age: 51, 53</td>
<td>Midway through treatments</td>
<td>Anxiety and depression were higher in chemo patients</td>
<td>So 2010</td>
</tr>
</tbody>
</table>

Abbreviations: RT: radiotherapy; HRT: hormone replacement therapy; HADS: Hospital Anxiety and Depression Scale; IDS-SR: Inventory of Depressive Symptomotology Self-Report; PSS: Perceived Stress Scale; PHQ-9: Patient Health Questionnaire 9 item; GAD-7: Generalized Anxiety 7 item Questionnaire; POMS: Profile of Mood States; BDI: Beck Depression Inventory; POMS-SF: Profile of Mood States Short Form; FACT-B: Functional Assessment of Cancer-Breast.
AC: adriamycin, cyclophosphamide; CAF: adriamycin, cyclophosphamide, 5-fluorouracil; CMF: cyclophosphamide, methotrexate, fluorouracil; ACT: adriamycin, cyclophosphamide, and a taxane; FEC: fluorouracil, epirubicin, cyclophosphamide; DC: docetaxil, cyclophosphamide; AP: adriamycin, paclitaxel; CFP: cyclophosphamide, 5-fluorouracil, paclitaxel.
### Table 9. Effects of chemotherapy on cognition in rodents

<table>
<thead>
<tr>
<th>Drug and dose</th>
<th>Route, duration, frequency</th>
<th>Animals (age if available)</th>
<th>Cognitive test (timing)</th>
<th>Cognitive outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doxorubicin (2.5mg/kg) + cyclophosphamide (25 mg/kg)</td>
<td>I.P. each week for 4 weeks</td>
<td>Female Sprague-Dawley rats (10mo.)</td>
<td>Passive avoidance (2 days after chemo)</td>
<td>Impaired learning</td>
<td>Konat 2008</td>
</tr>
<tr>
<td>Young-cyclo (100mg/kg) or 5-FU (150 mg/kg) Old- cyclo(80mg/kg)</td>
<td>I.P. every 4 weeks for 18 weeks (young) or 16 weeks (old)</td>
<td>Female Fischer rats young (7mo.) and old (18 mo.)</td>
<td>MWM + Stone 14-unit T-maze (young: 7 or 29 wks; old: 16 weeks post chemo)</td>
<td>Increased short term learning, no difference long term</td>
<td>Lee 2006</td>
</tr>
<tr>
<td>Cyclo (40mg/kg) + dox (4mg/kg)</td>
<td>I.V. (tail) 1/week for 3 weeks</td>
<td>Female ovariectomized Sprague-Dawley rats (eight weeks old)</td>
<td>Cued and contextual fear Conditioning (1 week post chemo)</td>
<td>Impaired contextual, but not cued, fear memory</td>
<td>Macleod 2007</td>
</tr>
<tr>
<td>Dox (.5, 2, or 8 mg/kg)</td>
<td>Single I.P. inj.</td>
<td>Male Wistar rats (180–350 g)</td>
<td>Inhibitory avoidance conditioning (3h, 24 h, 7 days)</td>
<td>Impairment of memory retention</td>
<td>Liedke 2009</td>
</tr>
<tr>
<td>Cyclophosphamide (8, 40, or 200 mg/kg)</td>
<td>Single I.P. injection</td>
<td>Male CF1 mice (70–90 days old)</td>
<td>Step-down inhibitory avoidance (1, 7 days)</td>
<td>Impaired inhibitory avoidance for 40 and 200 mg/kg</td>
<td>Reiriz 2006</td>
</tr>
<tr>
<td>Cyclophosphamide (40mg/kg)</td>
<td>Single I.P. injection</td>
<td>Male ICR mice (8–10 weeks old)</td>
<td>Passive avoidance + NOR (12 hrs. post injection)</td>
<td>Impaired passive avoidance learning</td>
<td>Yang 2010</td>
</tr>
<tr>
<td>Cyclo (30 mg/kg)</td>
<td>7 inj. (I.V. tail vein) every other day</td>
<td>Male Lister-hooded rats</td>
<td>NLR (5 days post chemo)</td>
<td>No diff. (working memory)</td>
<td>Lyons 2011</td>
</tr>
<tr>
<td>Dox (4mg/kg) OR 5-FU (100mg/kg) OR Dox and cyclo (4 and 80 mg/kg)</td>
<td>I.P. inj. 1/wk for 3 weeks</td>
<td>Female C57b./6J (9wks)</td>
<td>Context and cued fear conditioning; NOR (1-2 weeks after last treatment)</td>
<td>No difference</td>
<td>Fremouw 2012</td>
</tr>
<tr>
<td>Cyclo and 5-FU</td>
<td>Three I.P. inj. 1 month apart</td>
<td>Male F344 rats (5 mo.)</td>
<td>MWM, Fear conditioning, and 14-unit T maze (before and right after last treatment)</td>
<td>No impairment</td>
<td>Long 2011</td>
</tr>
<tr>
<td>5-FU (25 mg/kg)</td>
<td>I.V. (tail) every other day for 5 days</td>
<td>Male Lister-hooded rats (150–170 g)</td>
<td>Fear conditioning + NLR (day after treatment)</td>
<td>Impairment in both</td>
<td>Elbeltagy 2010</td>
</tr>
<tr>
<td>5-FU (20 mg/kg)</td>
<td>I.V. (tail vein) inj. 5 times across 12 days</td>
<td>Male Lister-hooded rats (200–250 g)</td>
<td>NLR</td>
<td>Impairment in NLR</td>
<td>Mustafa 2008</td>
</tr>
<tr>
<td>MTX + 5-FU; High dose: 37.5 MTX, 75 mg/kg 5-FU; Low dose: 38, 19 mg/kg</td>
<td>I.P. 1/wk for 4 weeks</td>
<td>Male C57BL/6Hsd mice (7-8 weeks old)</td>
<td>Contextual fear conditioning + NOR (6 weeks post chemo)</td>
<td>No memory impairment</td>
<td>Gandal 2008</td>
</tr>
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### Table 9 continued

<table>
<thead>
<tr>
<th>Drug and dose</th>
<th>Route, duration, frequency</th>
<th>Animals (age if available)</th>
<th>Cognitive test (timing)</th>
<th>Cognitive outcome</th>
<th>Reference</th>
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<tbody>
<tr>
<td>MTX (37.5 mg/kg) + 5-FU (75 mg/kg)</td>
<td>I.P. inj. 1/week for 3 weeks</td>
<td>Female BALB/C mice (2 mo.)</td>
<td>MWM, NMTS, dNMTS; 1 month after injection (1 wk post chemo)</td>
<td>Impairment in spatial memory but not cued memory or discrimination learning</td>
<td>Winocur 2006</td>
</tr>
<tr>
<td>MTX (50 mg/kg) + 5-FU (75 mg/kg)</td>
<td>I.P. inj. 1/wk for 4 weeks</td>
<td>Female balb/C (3 mo.)</td>
<td>MWM, NMTS and dNMTS tasks (1 wk post chemo)</td>
<td>Impairment in all tasks but cued memory</td>
<td>Winocur 2011</td>
</tr>
<tr>
<td>MTX (37.5 mg/kg) + 5-FU (50 mg/kg)</td>
<td>Single I.P. inj.</td>
<td>Female balb/C mice (5 mo.)</td>
<td>MWM, NMTS and DNMTS tasks (1 wk post chemo)</td>
<td>Impairment in all tasks but cued memory</td>
<td>Wincur 2012</td>
</tr>
<tr>
<td>Methotrexate (MTX) (250 mg/kg)</td>
<td>Single I.P. inj.</td>
<td>Male hooded Wistar rats (8 mo.)</td>
<td>MWM + NOR + go/no-go task (week 2 out to 8 mo.)</td>
<td>Impairment in all 3 tasks (visual, spatial, and discrimination learning)</td>
<td>Fardell 2010</td>
</tr>
<tr>
<td>MTX (adults 250 mg/kg; young 1 mg/kg)</td>
<td>Adults: single I.P. Young: 2/wk 1st two weeks, 1/wk for following 6 weeks</td>
<td>Male Long-Evans rats (12 weeks old) and young female and male (2 wks old)</td>
<td>NOR + NLR (3-7 days post chemo)</td>
<td>Impaired NLR, not NOR</td>
<td>Li 2010</td>
</tr>
<tr>
<td>MTX (1.5-6 mg/kg)</td>
<td>3 injections (1/day) in lateral ventricle</td>
<td>Male Wistar rats (four months old)</td>
<td>Conditioned avoidance Test (1 wk post chemo)</td>
<td>Impaired learning and memory</td>
<td>Madhyastha 2002</td>
</tr>
<tr>
<td>MTX (250 mg/kg)</td>
<td>Sing tail vein injection</td>
<td>Male Wistar rats (three months old)</td>
<td>MWM + NOR + contextual fear conditioning (2 wks)</td>
<td>Impairment in NOR, not MWM</td>
<td>Seigers 2009</td>
</tr>
<tr>
<td>MTX (0.005 mg/kg)</td>
<td>Single I.P. injection</td>
<td>Male and female Sprague-Dawley rats (17 days old).</td>
<td>Appetitive Pavlovian discrimination + conditioned taste aversion (at 80 days old)</td>
<td>No impairment</td>
<td>Stock 1995</td>
</tr>
<tr>
<td>Paclitaxel (1 mg/kg)</td>
<td>unclear</td>
<td>Male Long-Evans rats</td>
<td>5 choice serial reaction time task</td>
<td>No impairment (1-19 days post chemo)</td>
<td>Boyette-Davis 2009</td>
</tr>
<tr>
<td>Oxaliplatin (8 or 12 mg/kg) OR 75 mg/kg 5-FU OR combination of both drugs</td>
<td>Single I.P. inj.</td>
<td>Male hooded Wistar rats</td>
<td>NOR, MWM, Fear conditioning, context testing</td>
<td>Impairment in NOR, MWM, and context- all hippocampal related (2-3 wks post chemo)</td>
<td>Fardell 2012</td>
</tr>
<tr>
<td>thioTEPA (10 mg/kg)</td>
<td>I.P. daily for 3 days</td>
<td>Male C57BL/6J mice (five weeks old)</td>
<td>NOR + NLR 1-30 wks post inj.</td>
<td>Impairment in both at 12 and 20 weeks</td>
<td>Mondic 2010</td>
</tr>
<tr>
<td>Cytosine arabinoside (400 mg/kg)</td>
<td>I.P. injection every day for 5 days</td>
<td>Male Sprague-Dawley rats</td>
<td>MWM 7 days after last injection</td>
<td>Impairment in remote recall of MWM (1 wk post chemo)</td>
<td>Li 2008</td>
</tr>
</tbody>
</table>

Abbreviations: I.P.: Intraperitoneal; I.V.: Intravenous; MWM: Morris Water Maze; NOR: Novel Object Recognition; NLR: Novel Location Recognition; NMTS: non-matching to sample; DNMTS: delayed non-matching to sample; MTX: methotrexate.
**Table 10. Effects of chemotherapy on affective behavior in rodents**

<table>
<thead>
<tr>
<th>Drug and dose</th>
<th>Route, duration, frequency</th>
<th>Animals</th>
<th>Affect test</th>
<th>Affective outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doxorubicin (7 mg/kg)</td>
<td>Single I.P. inj.</td>
<td>Male Wistar rats</td>
<td>EPM and OF (1, 24, 48, and 73 hours after inj.)</td>
<td>Increased anxiety-like behavior, but decreased locomotor activity; myelosupression and increase in brain oxidative stress</td>
<td>Merzoug 2011</td>
</tr>
<tr>
<td>Doxorubicin (9 mg/kg)</td>
<td>Single dose I.V. (tail vein)</td>
<td>Pregnant C57bl/6 females injected, but offspring tested at 3 mo.</td>
<td>OF, passive avoidance, social exploration, EPM (age 12-14 weeks)</td>
<td>Increased anxiety (less time in open arms)</td>
<td>Van Calsteren 2009</td>
</tr>
<tr>
<td>Doxorubicin (10 mg/kg)</td>
<td>Single I.V. dose</td>
<td>Male Swiss albino mice</td>
<td>EPM and Vogel’s conflict test (8 and 14 days post chemo)</td>
<td>Increased anxiety-like behavior, reversed by alprazolam</td>
<td>Anwar 2011</td>
</tr>
<tr>
<td>Doxorubicin (10 mg/kg)</td>
<td>Single I.P. inj.</td>
<td>Female C57bl/6 (2 mo.)</td>
<td>TST and FST, EPM</td>
<td>Increased depressive-like behavior (FST, not TST), but not anxiety-like behavior</td>
<td>Kujo 2011</td>
</tr>
<tr>
<td>thioTEPA (10 mg/kg)</td>
<td>I.P. daily for 3 days</td>
<td>Male C57BL/6J mice (five weeks old)</td>
<td>FST 3, 6, 8, 13, and 21 weeks after inj. TST 8, 13, and 21 weeks after inj.</td>
<td>No effect on depressive behavior</td>
<td>Mondie 2010</td>
</tr>
<tr>
<td>Cyclophosphamide (8, 40, or 200 mg/kg)</td>
<td>Single I.P. injection</td>
<td>Male CF1 mice (70–90 days old)</td>
<td>OF (1, 7 days post chemo)</td>
<td>No effect on anxiety behavior</td>
<td>Reiriz 2006</td>
</tr>
<tr>
<td>Methotrexate + 5-FU</td>
<td>I.P. 1/wk for 4 weeks</td>
<td>Male C57BL/6Hsd mice (7-8 weeks old)</td>
<td>Contextual fear conditioning (6 weeks post chemo)</td>
<td>Increased anxiety-like behavior (increased freezing)</td>
<td>Gandal 2008</td>
</tr>
<tr>
<td>MTX (1.5-6 mg/kg)</td>
<td>3 injections (1/day) in lateral ventricle</td>
<td>Male Wistar rats (four months old)</td>
<td>Light/dark box (1 week post chemo)</td>
<td>No light/dark diff., but decreased exploratory behavior in dark Reduced brain monoamines</td>
<td>Madhyastha 2002</td>
</tr>
</tbody>
</table>

Abbreviations: MTX: methotrexate; EPM: elevated plus maze; OF: open field test; FST: forced swim test; TST: tail suspension test.
Table 11. Chemo induced inflammatory changes in clinical studies

<table>
<thead>
<tr>
<th>Study Type</th>
<th>Drug (cycles)</th>
<th>Population</th>
<th>Cytokine assessment</th>
<th>Neuropsychological/Behavioral assessment</th>
<th>Chemo effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cross-sectional</td>
<td>Not specified</td>
<td>n=103; post-chemo (48%), non-chemo (52%) Mean age: 51</td>
<td>Baseline#</td>
<td>Self report measures: FSI (fatigue), BDI-II (depression), and PSQI (sleep disturbance)</td>
<td>Chemo group had higher sTNF-rII, but not IL-1ra or CRP; sTNF-rII associated with fatigue in chemo group. Sleep disturbance and depression not associated with inflammatory markers</td>
<td>Bower et al 2011</td>
</tr>
<tr>
<td>Cross-sectional</td>
<td>AC, AP, CFP, or CMF</td>
<td>n=42 chemo BC patients n= 35 healthy controls</td>
<td>4.8±3.4 (range 1-12) years after treatment</td>
<td>HVLTR, WAIS-4 Self-report: MMQ (memory)</td>
<td>Chemo treated group had higher levels of TNF-a, IL-6, memory impairment, and reduced left hippocampal volume. These were all correlated with each other in chemo treated group, but not healthy controls.</td>
<td>Kessler 2013</td>
</tr>
<tr>
<td>Longitudinal</td>
<td>AC/CAF (n=27) Or CMF (n=27) Paxil (~50%/group)</td>
<td>BC patients n=27/group; Mean age: 53, 51</td>
<td>Prior to cycle 2 and after cycle 4</td>
<td>Self report (cognition): 5 questions from FSCL</td>
<td>IL-6 higher in AC/CAF group at 2nd timepoint than CMF; IL-6 increased across time in AC/CAF, but not CMF, group MCP-1 negatively corr. with self-report measures of thinking, concentration, and forgetfulness in AC/CAF but not CMF group</td>
<td>Janelisns 2012</td>
</tr>
<tr>
<td>Longitudinal</td>
<td>Not specified</td>
<td>n=33; BC patients Chemo n=23; non-chemo n=10 Mean age: 51, 56</td>
<td>Baseline# and 1 year later</td>
<td>Self report (cognition): PAOFI</td>
<td>Higher levels of circulating IL-1ra, sTNF-rII, and C reactive protein (CRP) at first time point in chemo group; higher CRP 1 year later Positive correlations across time between cytokines (IL-1ra, and sTNF-RII) and metabolism in medial frontal and temporal cortex No data reported relating cytokines to cognition</td>
<td>Pomykala 2013</td>
</tr>
<tr>
<td>Longitudinal</td>
<td>CMF or CAF with Paclitaxel or Docetaxel</td>
<td>BC patients N=15/group except for health controls (n=20)</td>
<td>Baseline# and after last cycle</td>
<td>N/A</td>
<td>At baseline, chemo groups had higher TNF-a and IL-6, and lower IL-2, IFN-y, and GM-CSF than healthy controls. Across time, chemo increased IL-6, GM-CSF, and IFN-y and decreased IL-1, TNF, and PGE2 levels. Change was greater for Paclitaxel than Docetaxel</td>
<td>Tsavaris 2002</td>
</tr>
</tbody>
</table>

Continued
<table>
<thead>
<tr>
<th>Study Type</th>
<th>Drug (cycles)</th>
<th>Population</th>
<th>Cytokine assessment</th>
<th>Neuropsychological/Behavioral assessment</th>
<th>Chemo effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Longitudinal</td>
<td>Paclitaxel or CAF</td>
<td>N=90 BC; 70 Paclitaxel, 20 FAC; 15 healthy controls</td>
<td>Baseline, day 3, and after last treatment</td>
<td>N/A</td>
<td>No differences at baseline. Across time IL-6, 8, and 10 was higher in Paclitaxel group than control. No diff. between FAC and control groups</td>
<td>Pusztai 2004</td>
</tr>
<tr>
<td>Longitudinal</td>
<td>ACT (29%), FEC (4%), DC (51%), taxC(18%), or GC (2%)</td>
<td>BC patients Chemo; n=49 Non-chemo; n=44</td>
<td>Baseline#, 6 months, 12 months after baseline</td>
<td>WTAR, CVLT-2, WMS-3, BVMT-R, ROCF, WAIS-3, TMT, Stroop, GP Self-report: FSI (fatigue), BDI-II (depression), PSIQ (sleep), SMQ (memory)</td>
<td>TNF-RII higher in chemo patients at baseline and 6 months, but not 1 year; TNF-RII associated with memory complaints in chemo, but not non-chemo patients. TNF-RII is higher in chemo group and is associated with diminished metabolism in inferior frontal cortex. No relationship between baseline cytokines and cognitive functioning. No chemo effect on cognitive functioning at baseline; chemo had lower psychomotor functioning, but not significant (p=.07)</td>
<td>Ganz 2013</td>
</tr>
<tr>
<td>Longitudinal</td>
<td>Not specified</td>
<td>BC patients Chemo; n=24 Non-chemo; n=40</td>
<td>1 week before, week 6 of, and 6 weeks after RT; Chemo received before RT.</td>
<td>Self-report: IDS-SR (depression), MFI (fatigue), PSS (distress)</td>
<td>Chemotherapy was associated with higher levels of NF-kB DNA binding in peripheral blood mononuclear cells, IL-6, and sTNF-R2 as well as depression and fatigue. Chemotherapy and NF-kB DNA binding were sig. predictors of depression.</td>
<td>Torres 2013</td>
</tr>
</tbody>
</table>

#post-chemotherapy; £ post surgery and pre-chemo; §pre-surgery and pre-chemo
AC: adriamycin, cyclophosphamide; CAF: adriamycin, cyclophosphamide, 5-fluorouracil; CMF: cyclophosphamide, methotrexate, fluorouracil; ACT: adriamycin, cyclophosphamide, and a taxane; FEC: fluorouracil, epirubicin, cyclophosphamide; DC: docetaxil, cyclophosphamide; taxC: a taxane and carboplatin; GC: gemcitabine, cisplatin; AP: adriamycin, paclitaxel; CFP: cyclophosphamide, 5-fluorouracil, paclitaxel
<table>
<thead>
<tr>
<th>Study Type</th>
<th>Imaging Technique</th>
<th>Drug</th>
<th>Population</th>
<th>Cognitive Tests</th>
<th>Testing Period</th>
<th>Chemo effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cross-sectional</td>
<td>MRI</td>
<td>AC CMF, EC PTX, UFT</td>
<td>n=106; post-chemo (51), non-chemo (55) n= 55 healthy controls Mean age: 46</td>
<td>WMS-R</td>
<td>Baseline#</td>
<td>Chemo group had smaller gray and white matter in prefrontal, parahippocampal, and cingulated gyrus. These correlated with attention, concentration, and visual memory</td>
<td>Inagaki 2007</td>
</tr>
<tr>
<td>Cross-sectional</td>
<td>MRI</td>
<td>AC CMF, EC PTX, 5-FU, DFUR, HCFU, UFT</td>
<td>n=132; post-chemo (73), non-chemo (59) n= 37 healthy controls Mean age: 48</td>
<td>WMS-R</td>
<td>Baseline#</td>
<td>No difference</td>
<td>Inagaki 2007</td>
</tr>
<tr>
<td>Cross-sectional</td>
<td>MRI</td>
<td>FEC + CTC</td>
<td>n=32; post-chemo (17), non-chemo (15) n= 10 healthy controls Mean age: 57, 58</td>
<td>Digit Symbol test</td>
<td>Average 22 months post chemotherapy</td>
<td>Chemo patients had lower white matter integrity in the genu of the CC, associated with slower processing speed</td>
<td>de Ruiter, 2011</td>
</tr>
<tr>
<td>Cross-sectional</td>
<td>MRI</td>
<td>AC, AC+taxane</td>
<td>n=75; chemo (44), non-chemo (31) Mean age: 48,48</td>
<td>WMS-R</td>
<td>More than 3 years post chemo</td>
<td>No difference in hippocampal volume or memory ability</td>
<td>Yoshikawa2005</td>
</tr>
<tr>
<td>Cross-sectional</td>
<td>MRI</td>
<td>FEC, FEC+Taxol</td>
<td>n=17 post-chemo n= 18 healthy controls Mean age: 57, 58</td>
<td>BWD, TEA, WAIS, AVL, RVDLT, SCWT, COWA, 9HPT, TMT; Self report: CFQ</td>
<td>80-160 days post chemo</td>
<td>Chemo group had reduced white matter integrity which correlated with attention and processing/psychomotor speed</td>
<td>Deprez 2011</td>
</tr>
<tr>
<td>Cross-sectional</td>
<td>MRI</td>
<td>AC, AT, CFP, CMF</td>
<td>n=42 post-chemo n= 35 healthy controls Mean age: 55, 56</td>
<td>WAIS4</td>
<td>Average 5 years post chemo (range 1-12)</td>
<td>Chemo group had reduced left hippocampal volume which was associated with verbal memory performance.</td>
<td>Kesler 2013</td>
</tr>
<tr>
<td>Cross-sectional</td>
<td>MRI</td>
<td>CMF</td>
<td>n= 184 chemo n= 368 healthy controls</td>
<td>-</td>
<td>Average 21 years post treatment</td>
<td>Chemothrapy treated patients had smaller total brain volume and gray matter volume; no difference in hippocampal volume or white matter.</td>
<td>Koppelmans 2012</td>
</tr>
<tr>
<td>Study Type</td>
<td>Imaging Technique</td>
<td>Drug</td>
<td>Population</td>
<td>Cognitive Tests</td>
<td>Testing Period</td>
<td>Chemo effect</td>
<td>Reference</td>
</tr>
<tr>
<td>------------------</td>
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</tr>
<tr>
<td>Longitudinal</td>
<td>MRI</td>
<td>AC, ACT, taxol+carboplatin, taxol + cisplatin</td>
<td>n=28; chemo (27), non-chemo (28) n= 24 healthy controls Mean age: 50,52,47</td>
<td>Self-report: BRIEF</td>
<td>Baseline and 1 month post treatment</td>
<td>Chemo patients, but not non-chemo or healthy controls, had decreased frontal gray matter after treatment which was associated with executive functioning difficulties</td>
<td>McDonald 2013</td>
</tr>
<tr>
<td>Longitudinal</td>
<td>MRI</td>
<td>AC, AC+taxol</td>
<td>n=106; chemo (17), non-chemo (12) n= 18 healthy controls Mean age: 46</td>
<td>-</td>
<td>Baseline</td>
<td>No difference at baseline. Chemo had decreased gray matter density at 1 and 12 months after treatment. No difference in non-chemo or control groups.</td>
<td>McDonald 2010</td>
</tr>
<tr>
<td>Longitudinal</td>
<td>MRI</td>
<td>Unspecified</td>
<td>n=50; chemo (34), non-chemo (16) n= 19 healthy controls Mean age: 44,43,44</td>
<td>not completely specified</td>
<td>Baseline and 3-4 months post treatment</td>
<td>Chemo patients had decreased white matter integrity across time; non-chemo and healthy controls did not. Attention and verbal ability correlated with white matter integrity changes.</td>
<td>Deprez 2012</td>
</tr>
<tr>
<td>Cross-sectional</td>
<td>fMRI</td>
<td>AC, ACT, CarT, CT, FECT, CMF, ACF</td>
<td>n=28; chemo (25), non-chemo (19) n= 18 healthy controls Mean age: 56,58,56</td>
<td>WCST, WAIS4 digit span, DKEFS, Self-report: BRIEF</td>
<td>Average 5 years post treatment</td>
<td>Chemo patients had reduced activation in left caudal lateral prefrontal cortex compared to non-chemo and healthy controls. This was sig. correlated with executive dysfunction in the chemo group.</td>
<td>Kesler 2011</td>
</tr>
<tr>
<td>Cross-sectional</td>
<td>fMRI</td>
<td>CMF, ACT</td>
<td>n = 14 post-chemo n= 14 healthy controls Mean age: 55,54</td>
<td>Verbal memory encoding and recall tasks</td>
<td>Average 3 years post treatment</td>
<td>During memory encoding, chemotherapy patients had lower activation than controls and CMF treated patients had lower prefrontal cortex activation than ACT and controls. Chemo had higher activation in multiple brain regions during memory compared to healthy controls.</td>
<td>Kessler 2009</td>
</tr>
<tr>
<td>Cross-sectional</td>
<td>fMRI</td>
<td>FEC + CTC</td>
<td>n=34; chemo (19), non-chemo (15) Mean age: 56, 58</td>
<td>TMT, WAIS-III DSCT, Stroop, CVLT, WMS-R VRT, FFT, WFT, tower of London, paired associates task</td>
<td>9 years after chemo</td>
<td>Chemo group showed less activity in the dorsolateral prefrontal cortex during executive functioning task, parahippocampal gyrus during the memory task, and bilateral posterior parietal cortex during both tasks. Chemo patients showed cognitive deficits in verbal memory and planning</td>
<td>de Ruiter 2011</td>
</tr>
<tr>
<td>Study Type</td>
<td>Imaging Technique</td>
<td>Drug</td>
<td>Population</td>
<td>Cognitive Tests</td>
<td>Testing Period</td>
<td>Chemo effect</td>
<td>Reference</td>
</tr>
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</tr>
<tr>
<td>Longitudinal</td>
<td>fMRI</td>
<td>FECT, FECTH, CT, AC</td>
<td>n=21 post-chemo; n= 21 healthy controls Mean age: 50, 50</td>
<td>Verbal recall task</td>
<td>Baseline and 1 month post treatment</td>
<td>Chemo group showed less anterior cingulated activation at baseline than healthy controls. Across time chemo patients had less activation in multiple areas and had less activation than controls; no change across time in controls</td>
<td>Zunini 2012</td>
</tr>
<tr>
<td>Longitudinal</td>
<td>fMRI</td>
<td>AC, ACT</td>
<td>n=28; chemo (16), non-chemo (12); n= 15 healthy controls Mean age: 53,53,51</td>
<td>n-back task</td>
<td>Baseline, 1 month and 1 year post treatment</td>
<td>Both cancer groups showed increased bifrontal and decreased left parietal activation at baseline and decreased frontal hyperactivation 1 month post treatment compared to healthy controls. Chemo effect on frontal lobe 1 month after chemo.</td>
<td>McDonald, Conroy, Ahles 2012</td>
</tr>
<tr>
<td>Cross-sectional</td>
<td>qEEG</td>
<td>FEC or FEC+ high dose CTC</td>
<td>n=47; high dose chemo (17), std. dose (16), non-chemo (14) Mean age: 46,49,49</td>
<td>Not specified but gave a ref.</td>
<td>Average 1.5-2 years post chemo</td>
<td>Asymmetry of alpha rhythm in 41% of high dose and 12% of standard dose patients compared to 0% in non-chemo controls.</td>
<td>Schagen 2001</td>
</tr>
<tr>
<td>Cross-sectional</td>
<td>PET</td>
<td>Not specified</td>
<td>n=16 post-chemo; n= 8 controls; non-chemo (5), healthy (3) Mean age: 50, 53</td>
<td>21 neuropsychological tests (not specified but gave a ref.)</td>
<td>5-10 yrs post chemo</td>
<td>Chemo group showed altered cerebral blood flow during short term memory recall task relative to control group. Metabolism in basal ganglia was decreased in tamoxifen chemo group relative to chemo w/out tamoxifen or controls</td>
<td>Silverman 2007</td>
</tr>
<tr>
<td>Case study</td>
<td>MRI and fMRI</td>
<td>ACT</td>
<td>n=2 (monozygotic twins); chemo (1), non-chemo (1) Age: 60</td>
<td>n-back task</td>
<td>22 months post chemo</td>
<td>Chemo patient had increased white matter (WM) lesions in both cerebral hemispheres and increased brain activity during working memory task; No WM volume differences in other brain regions.</td>
<td>Ferguson 2007</td>
</tr>
</tbody>
</table>

#post-chemotherapy; £ post surgery and pre-chemo; §pre-surgery and pre-chemo
AC: adriamyacin, cyclophosphamide; CMF: cyclophosphamide, methotrexate, fluorouracil; EC: epirubicin, cyclophosphamide PTX: paclitaxel; UFT: tegafur/uracil; 5-FU: 5-fluorouracil; 5-DFUR: doxifluridine; HCFU: carmofur; ACT: adriamyacin, cyclophosphamide, taxol or taxotere; FECT: fluorouracil, epirubicin, cyclophosphamide, taxol; FECTH: FECT + hepirubicin; CT: cyclophosphamide, taxol; CAR: carmofur; MRI: magnetic resonance imaging; fMRI: functional MRI; PET: positron emission tomography; qEEG: quantitative electroencephalography.
<table>
<thead>
<tr>
<th>Drug/Dose</th>
<th>Mechanism</th>
<th>Route/ frequency/duration</th>
<th>Animals/cells</th>
<th>Outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doxorubicin (20mg/kg)</td>
<td>Apoptosis, Inflammation</td>
<td>Single i.p. inj. (72 hours)</td>
<td>Male B6C3 (2 mo.) mice</td>
<td>Dox increased TNFa, pro-apoptotic proteins, and cell death; xanthone derivative abolished these effects</td>
<td>Tangpong 2011</td>
</tr>
<tr>
<td>Doxorubicin (0.01-1 mg/mL)</td>
<td>Inflammation, Neurotransmitter</td>
<td>0-24 hours after treatment</td>
<td>Human Urothelial cells (RT4)</td>
<td>Dox increased IL-8, IL-1b, PGE2, and basal ACH</td>
<td>Kang 2013</td>
</tr>
<tr>
<td>Doxorubicin (20mg/kg)</td>
<td>Apoptosis, Inflammation, Oxidative Stress</td>
<td>Single i.p. injection (3 hours)</td>
<td>Male B6C3 mice (2 mo.)</td>
<td>Dox increased TNFa in brain, pro-apoptotic proteins, and induced mitochondrial dysfunction; inhibiting TNFa abolished these effects</td>
<td>Tangpong 2006</td>
</tr>
<tr>
<td>Doxorubicin (20mg/kg)</td>
<td>Inflammation, Oxidative Stress</td>
<td>Single i.p. inj. (3 hour treatment)</td>
<td>Male B6C3 and iNOSKO</td>
<td>Dox increased TNFa in WT and iNOSKO and decreased mitochondrial respiration in WT, but not iNOSKO mice. Dox also reduced MnSOD, a mitochondrial antioxidant enzyme</td>
<td>Tangpong 2007</td>
</tr>
<tr>
<td>Doxorubicin (45 mg/kg)</td>
<td>Oxidative Stress</td>
<td>Single i.v. (48 hours)</td>
<td>Male Wistar rats</td>
<td>Dox increased MDA, decreased GSH; melatonin abolished these effects</td>
<td>Oz 2006</td>
</tr>
<tr>
<td>Doxorubicin (20 mg/kg)</td>
<td>Oxidative Stress</td>
<td>Single i.p. inj. (72 hrs.)</td>
<td>Male mice (2-3 mo.)</td>
<td>Dox increases oxidative stress (protein oxidation, lipid peroxidation, MRP1 expression)</td>
<td>Joshi 2005</td>
</tr>
<tr>
<td>Doxorubicin (20 mg/kg)</td>
<td>Oxidative Stress</td>
<td>Single i.p. inj. (72 hrs.)</td>
<td>Male B6C3 mice (2-3 mo.)</td>
<td>Dox increases oxidative stress (protein oxidation, lipid peroxidation, MRP1 expression) and decreased glutathione (GSH). GCEE reversed these effects.</td>
<td>Joshi 2007</td>
</tr>
<tr>
<td>Doxorubicin (20 mg/kg)</td>
<td>Oxidative Stress</td>
<td>ADR: i.p. inj. (72hr. before tissue collection)</td>
<td>Male B6C3 mice (2-3 mo.)</td>
<td>Dox decreased GSH and increased GPx, GST, and GR levels. Activity levels were increased for GPx and decreased for GST and GR. Dox increased oxidation of peroxiredoxen 1, TPI, and enolase proteins</td>
<td>Joshi 2010</td>
</tr>
<tr>
<td>Doxorubicin (25 mg/kg)</td>
<td>Oxidative Stress</td>
<td>Single inj.</td>
<td>Male B6C3 mice</td>
<td>Dox increases oxidative stress and TNFa. 2-Mercaptoethane sulfonate prevents these effects.</td>
<td>Aluise 2011</td>
</tr>
<tr>
<td>Cyclophosphamide (50 mg/kg), 5-FU (60 mg/kg), dox (5 mg/kg), paclitaxel (5 mg/kg)</td>
<td>Neurogenesis/ gliogenesis</td>
<td>3 i.p. injections at day 1,4,7 (tissue collected 48 hours later)</td>
<td>C57BL/6 (2 mo.)</td>
<td>All drugs reduced neural cell proliferation, but not apoptosis, in the dentate gyrus. Insulin-like growth factor (IGF-1) slightly reduced this effect in mice treated with cyclo.</td>
<td>Janelinsins 2010</td>
</tr>
</tbody>
</table>

Continued
Table 13 continued

<table>
<thead>
<tr>
<th>Drug/Dose</th>
<th>Mechanism</th>
<th>Route/ frequency/duration</th>
<th>Animals/cells</th>
<th>Outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclophosphamide (30 mg/kg)</td>
<td>Neurogenesis/ gliogenesis</td>
<td>1 i.v. doses every other day for 7 treatments</td>
<td>Male Lister-hooded rats</td>
<td>Chemo reduced brdU (survival of hippocampal cells), but not Ki67 (proliferation); No difference in NLR behavior</td>
<td>Lyons 2011</td>
</tr>
<tr>
<td>Cyclophosphamide (40 mg/kg)</td>
<td>Neurogenesis</td>
<td>Single i.p. inj.</td>
<td>Male ICR mice (2 mo.)</td>
<td>CP impairs learning/memory (NOR and passive avoidance) and decreases neurogenesis for up to one week</td>
<td>Yang 2010</td>
</tr>
<tr>
<td>Cyclophosphamide (40mg/kg), 5-FU (75 mg/kg), and MTX (37.5 mg/kg)</td>
<td>Histone acetylation, Neurogenesis</td>
<td>I.P. 1/week for 4 weeks (behavior 2 weeks after last treatment)</td>
<td>Female Wistar rats (4 mo.)</td>
<td>CMF decreased hipp. cell proliferation and histone deacetylase activity, impaired learning/memory (MWM), and increased histone acetylation</td>
<td>Briones 2011</td>
</tr>
<tr>
<td>Cyclophosphamide (50mg/kg) and doxorubicin (2 mg/kg)</td>
<td>Inflammation</td>
<td>Four i.p. inj. 1/week (1 week before behavior)</td>
<td>Male athymic nude rats (2 mo.)</td>
<td>Both agents reduced neurogenesis and impaired learning/memory (NLR and fear conditioning). CP, not DOX, activated microglia (ED-1+)</td>
<td>Christie 2012</td>
</tr>
<tr>
<td>Cyclophosphamide (75 mg/kg)</td>
<td>Oxidative Stress</td>
<td>Single i.p. inj. (6-8 weeks)</td>
<td>Male Swiss albino mice (6-8 weeks)</td>
<td>CP increases oxidative stress (decreases GSH, GSH peroxidase, and alkaline phosphatase). Melatonin reversed these effects.</td>
<td>Manda 2003</td>
</tr>
<tr>
<td>Cyclophosphamide (75mg/kg)</td>
<td>Oxidative Stress</td>
<td>Single i.p. inj. (24 hours)</td>
<td>Wistar albino rats</td>
<td>CP increased MDA in brain; reduced by hot short pepper</td>
<td>Oboh 2010</td>
</tr>
<tr>
<td>Cyclophosphamide (75mg/kg)</td>
<td>Oxidative Stress</td>
<td>Single i.p. inj. (24 hours)</td>
<td>Wistar albino rats</td>
<td>CP increased MDA, AST, ALT levels; reduced by yellow dye extract from Brimstone tree root</td>
<td>Oboh 2012</td>
</tr>
<tr>
<td>Cyclophosphamide (75mg/kg)</td>
<td>Oxidative Stress</td>
<td>Single i.p. inj. (24 hours)</td>
<td>Wistar albino rats</td>
<td>CP increased lipid peroxidation (increased MDA levels); reduced by red dye extract from Sorghum stem</td>
<td>Oboh 2010</td>
</tr>
<tr>
<td>Cyclophosphamide (75 mg/kg)</td>
<td>Oxidative Stress</td>
<td>Single i.p. inj. (24 hrs.)</td>
<td>Male Swiss mice (2 mo.)</td>
<td>CP increased oxidative stress; effects reduced by Linseed oil.</td>
<td>Bhatia 2006</td>
</tr>
</tbody>
</table>

Continued
<table>
<thead>
<tr>
<th>Drug/Dose</th>
<th>Mechanism</th>
<th>Route/ frequency/duration</th>
<th>Animals/cells</th>
<th>Outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cisplatin, Cyclophosphamide, methotrexate, vinblastin, thioTEPA</td>
<td>Apoptosis, Morphology</td>
<td>In-vitro: (24 hour treatment) In-vivo: i.p. injection (4-24 hours before tissue collection)</td>
<td>In-vitro: neurons from 19-day old male Wistar rats In-vivo: 7 day old Wistar rats</td>
<td>In-vivo, all drugs induced neurotoxicity and cell death. In-vitro, chemo drugs induced neurotoxicity, which were ameliorated by N-methyl-D-aspartate receptor MK801, AMPAra GYKI52466, and pancaspase inhibitor Ac-DEVD-CHO</td>
<td>Rzeski 2004</td>
</tr>
<tr>
<td>Methotrexate (0.2 mg/kg)</td>
<td>Oxidative Stress</td>
<td>Daily subcutaneous inj. for 7 days (analysis performed 2 days after last inj.)</td>
<td>Male Wistar rats</td>
<td>MTX increased oxidative stress (decreased GSH, increased protein carbonyls, SOD, and catalase)</td>
<td>Rajamani 2006</td>
</tr>
<tr>
<td>Methotrexate (75 mg/kg)</td>
<td>Neurogenesis/ gliogenesis</td>
<td>2 i.v. doses 1 week apart</td>
<td>Male Lister-hooded rats</td>
<td>MTX reduced hippoc. cell survival, proliferation, and NLR behavior; Fluoxetine prevented these effects</td>
<td>Lyons 2011</td>
</tr>
<tr>
<td>Methotrexate (37.5-300mg/kg)</td>
<td>Neurogenesis/ gliogenesis</td>
<td>Single i.v. inj.</td>
<td>Male Wistar rats (3 mo.)</td>
<td>MTX reduced cell proliferation and showed memory impairment in NLR and MWM probe latency, but not MWM training</td>
<td>Seigers 2008</td>
</tr>
<tr>
<td>MTX (250 mg/kg)</td>
<td>Neurogenesis/ gliogenesis</td>
<td>Sing tail vein injection</td>
<td>Male Wistar rats (3 mo.)</td>
<td>MTX reduced hippoc. cell proliferation 7 days after treatment, but not 1 day after; No diff. in cell death</td>
<td>Seigers 2009</td>
</tr>
<tr>
<td>Methotrexate (100 mg/kg)</td>
<td>Neurogenesis/ gliogenesis</td>
<td>Single i.p. inj.</td>
<td>Male buffalo rats (3 mo.)</td>
<td>MTX reduces hippocampal cell proliferation whereas having a tumor did not</td>
<td>Seigers 2010</td>
</tr>
<tr>
<td>Methotrexate (40 mg/kg)</td>
<td>Neurogenesis/ gliogenesis, oxidative stress</td>
<td>Single i.p. inj. (24 hr. prior to behavior)</td>
<td>C3H/HeN tumor bearing mice (6 weeks)</td>
<td>MTX increased depressive-like behavior (TST) and cognitive impairment (passive avoidance), reduced hippoc. neurogenesis, and upregulated iNOS and COX-2 enzymes</td>
<td>Yang 2012</td>
</tr>
<tr>
<td>MTX</td>
<td>Apoptosis, Neurogenesis/ gliogenesis</td>
<td>In-vitro: 24 hour treatment In-vivo: Single i.p. inj. (6 hours to 14 days)</td>
<td>In-vitro: embryonic rat hippocampal cells In-vivo:Male 57BL/6 (2 mo.)</td>
<td>MTX-treated mice showed increased depressive-like behavior (TST) and memory deficits (NOR) in addition to increased apoptosis and decreased cell proliferation in the hippocampus</td>
<td>Yang 2011</td>
</tr>
</tbody>
</table>

Continued
### Table 13 continued

<table>
<thead>
<tr>
<th>Drug/Dose</th>
<th>Mechanism</th>
<th>Route/ frequency/duration</th>
<th>Animals/cells</th>
<th>Outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methotrexate + 5-FU</td>
<td></td>
<td></td>
<td></td>
<td>EEG gating ratio was higher in both chemo groups at week 5; no difference ERP amplitude or latency of P1</td>
<td>Gandal 2008</td>
</tr>
<tr>
<td>High dose: 37, 75 mg/kg</td>
<td>Electrophysiology</td>
<td>I.P. 1/wk for 4 weeks</td>
<td>Male C57BL/6Hsd mice (7-8 wk old)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low dose: 38, 19 mg/kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MTX (0.5mg/kg)</td>
<td>CSF Composition</td>
<td></td>
<td>Male Long Evans rats (8 weeks)</td>
<td>MTX induced deficits in spatial and visual memory (NOR and NLR)</td>
<td>Vijayanathan 2011</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>MTX increased homocysteine sulfonic acid and homocysteic acid in CSF, briefly in single inj. group and out to 3 months in multiple inj. group</td>
<td></td>
</tr>
<tr>
<td>MTX (0.5mg/kg)</td>
<td>CSF Composition</td>
<td></td>
<td>Male Wistar rats (3 mo.)</td>
<td>MTX decreased folate and increased homocysteine in CSF. MTX induced deficits in spatial and visual memory (NOR and NLR)</td>
<td></td>
</tr>
<tr>
<td>MTX</td>
<td></td>
<td></td>
<td></td>
<td>Acute and chronic MTX impaired spatial memory (NLR) but not visual memory (NOR) deficits and reduced folate in serum and CSF.</td>
<td>Li 2010 Intrathecal</td>
</tr>
<tr>
<td>Acute: 250 mg/kg</td>
<td>CSF Composition</td>
<td>Acute: single i.p. inj. (3 days before behave.)</td>
<td>Male Long Evan rats (10 weeks)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic: 1mg/kg</td>
<td>CSF Composition</td>
<td>Chronic: 10 I.P. inj. over 8 weeks (10 weeks after first inj.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MTX (250 mg/kg)</td>
<td>Blood Supply</td>
<td>Single i.v. inj. (1 or 3 weeks)</td>
<td>Male Wistar rats (3 mo.)</td>
<td>MTX reduced hipp blood vessel density 1 and 3 weeks after treatment and metabolism (via PET) at 1 but not 3 weeks; MTX reduced peripheral, but not hippocampal inflammatory cytokines, activated microglia at 1, 3 weeks.</td>
<td>Seigers 2010</td>
</tr>
<tr>
<td>MTX (1-2 mg/kg)</td>
<td>Neurotransmitter/monooamine release</td>
<td>1-5 i.c.v. inj. every 3 days for those receiving more than 1 inj.</td>
<td>Male Sprague-Dawley rats (4 weeks)</td>
<td>Single dose of MTX reduced metabolites of dopamine, but not serotonin 6 hours after inj. Multiple injections resulted in increased serotonin metabolites; no diff. on dopamine</td>
<td>Silverstein 1986</td>
</tr>
<tr>
<td>MTX (1.5-6 mg/kg)</td>
<td>Neurotransmitter/monooamine release</td>
<td>3 daily injections in lateral ventricle (1 week before behavior)</td>
<td>Male Wistar rats (4 mo.)</td>
<td>MTX reduced brain amines (NorE, Dop, Ser), increased hipp. neuronal death, and induced learning deficits (conditioned avoidance test), but did not affect anxiety-like behavior (light dark box)</td>
<td>Madhyasta 2002</td>
</tr>
<tr>
<td>5-FU (40 mg/kg)</td>
<td>Apoptosis, Inflammation, Electrophysiology, Neurogenesis/Gliogenesis</td>
<td>In-vitro: 0.5-5uM for 24hours to 5 days In-vivo: 3 i.p. inj. one every other day (1,7,14,56 days or 6 months)</td>
<td>In-vitro: purified CNS cells from developing rat; cancer cell lines In-vivo: CBA mice (6-8 weeks)</td>
<td>In-vitro: 5-FU killed CNS cells but not cancer cells In-vivo: 5-FU increased apoptosis, decreased proliferation and induced delayed damage to myelinated tracts and auditory functioning; No difference in microglial activation (F4/80 marker)</td>
<td>Han 2008</td>
</tr>
</tbody>
</table>

Continued
Table 13 continued

<table>
<thead>
<tr>
<th>Drug/Dose</th>
<th>Mechanism</th>
<th>Route/ frequency/duration</th>
<th>Animals/cells</th>
<th>Outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-FU (25 mg/kg)</td>
<td>Neurogenesis/ gliogenesis</td>
<td>i.v. inj. every other day for 5 total injections</td>
<td>Male Lister hooded rats</td>
<td>5-FU impaired working memory (NLR and conditioned emotional response test(CER)) and reduced proliferation in dentate gyrus. Fluoxetine, an anti-depressant improved NLR and proliferation relative to 5-FU group</td>
<td>ElBeltagy 2010</td>
</tr>
<tr>
<td>5-FU (25 mg/kg)</td>
<td>Neurogenesis/ gliogenesis</td>
<td>i.v. inj. every other day for 6 total injections</td>
<td>Male Lister hooded rats</td>
<td>5-FU reduced cell proliferation in dentate gyrus 2-4 weeks after, but not immediately following last treatment. COX-2 (inflammation) not corr. w/ cell proliferation or survival</td>
<td>ElBeltagy 2010</td>
</tr>
<tr>
<td>5-FU (25 mg/kg)</td>
<td>Neurogenesis/ gliogenesis</td>
<td>5 inj. over 2 weeks</td>
<td>Male Lister hooded rats</td>
<td>5-FU reduced hipp. cell survival, proliferation, and NLR behavior; Fluoxetine reversed these effects</td>
<td>Lyons 2012</td>
</tr>
<tr>
<td>5-FU (20 mg/kg)</td>
<td>Neurogenesis/ gliogenesis</td>
<td>5 i.v. inj. within 12 days</td>
<td>Male Lister-hooded rats</td>
<td>5-FU reduced BDNF and doublecortin levels in the hippocampus; no effect on spatial working memory (NLR)</td>
<td>Mustafa 2008</td>
</tr>
<tr>
<td>ThioTEPA (1,5, or 10 mg/kg)</td>
<td>Neurogenesis/ gliogenesis</td>
<td>I.P. inj. daily for 3 days</td>
<td>C57BL/6 mice (6 wks.) old</td>
<td>ThioTEPA, but not 5-FU, reduces cell proliferation in the dentate gyrus of the hippocampus</td>
<td>Mignone 2006</td>
</tr>
<tr>
<td>ThioTEPA (10 mg/kg)</td>
<td>Neurogenesis/ gliogenesis</td>
<td>I.P. inj. daily for 3 days</td>
<td>Male C57BL/6 (8 weeks)</td>
<td>ThioTEPA reduced cell proliferation; learning (NOR) was impaired at 12 and 20 weeks, but not earlier; no effect in Porsolt or TST behavior</td>
<td>Mondie 2010</td>
</tr>
<tr>
<td>ThioTEPA (10mg/kg)</td>
<td>Neurogenesis/ gliogenesis</td>
<td>Three daily i..p. inj.</td>
<td>Male C57BL/6 (2 mo.)</td>
<td>ThioTEPA reduced cell proliferation and increased depressive-like behavior in the sucrose anhedonia but not the Porsolt test.</td>
<td>Wilson 2013</td>
</tr>
<tr>
<td>Cytosine arabinoside (AraC; 400 mg/kg)</td>
<td>Morphology</td>
<td>I.P. inj. for 5 consecutive days (1 day before behavior)</td>
<td>Male Wistar rats (10 weeks old)</td>
<td>AraC impaired motor behavior (footprint pattern and rotarod) but did not affect learning (MWM). AraC also reduced cerebellar neurofilament activity.</td>
<td>Koros 2007</td>
</tr>
<tr>
<td>AraC (AraC; 400 mg/kg)</td>
<td>Morphology</td>
<td>I.P. inj. for 5 consecutive days (tissue collection 24 hours after last inj.)</td>
<td>Male Wistar rats (10 weeks old)</td>
<td>AraC reduced neurofilament isoform H in the cerebellum. N-acetylcysteine (NAC), an antioxidant prevented most of this reduction.</td>
<td>Koros 2009</td>
</tr>
<tr>
<td>AraC (400 mg/kg)</td>
<td>Morphology</td>
<td>I.P. inj. for 5 consecutive days (tissue collection 24 hours after last inj.)</td>
<td>Male Sprague-Dawley rats</td>
<td>AraC treatment resulted in impaired long-term, but not short-term memory (MWM) and a reduced dendritic length, spine density and number of branch points in anterior cingulated cortex, but not hippocampal CA1.</td>
<td>Li 2008</td>
</tr>
<tr>
<td>Drug/Dose</td>
<td>Mechanism</td>
<td>Route/ frequency/duration</td>
<td>Animals/cells</td>
<td>Outcome</td>
<td>Reference</td>
</tr>
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<td>-----------------------------------------------</td>
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<td>----------------------------------------------------</td>
<td>--------------------------------------------------------------------------------------------------</td>
<td>---------------------</td>
</tr>
<tr>
<td>Carmustine (BCNU; 1uM)</td>
<td>Neurogenesis/ gliogenesis</td>
<td>Single treatment (1 hour treatment)</td>
<td>Oligodendrocyte precursor cells (O-2A/OPC)</td>
<td>BCNU disrupted O-2A/OPC cell division and differentiation, increasing cell cycle length and decreasing time to differentiation and likelihood of self-renewal</td>
<td>Hyrien 2010</td>
</tr>
<tr>
<td>Carmustine (BCNU), cisplatin, cytarabine</td>
<td>Apoptosis, neurogenesis/ gliogenesis</td>
<td>In-vitro: BCNU 1 hour; cisplatin 20 hrs.; cytarabine 24 hours; In-vivo: 3 consecutive i.p. injections</td>
<td>In-vitro: neuroepithelial cells from rat spinal cord; tumor cell lines In-vivo: CBA mice (6-8 weeks)</td>
<td>Chemo agents increased cell death, suppressed cell division In-vitro: chemotherapy agents are more toxic for CNS progenitor cells and nondividing oligodendrocytes than cancer cell lines</td>
<td>Dietrich 2006</td>
</tr>
<tr>
<td>BCNU (20mg/kg)</td>
<td>Apoptosis, Oxidative stress</td>
<td>Single i.v. injection (3 weeks before behavior)</td>
<td>60 male Wistar rats</td>
<td>BCNU increases TNFa, apoptosis, oxidative stress, cog. deficits; Metallothionein (i.c.v.) reduces these effects</td>
<td>Helal 2009</td>
</tr>
<tr>
<td>Cisplatin (0.1, .5, or 1 mg/kg)</td>
<td>Electrophysiology</td>
<td>Daily i.p. inj. for 3 consecutive days</td>
<td>Male Sprague-Sawley rats</td>
<td>High dose increased thermal and mechanical withdrawal thresholds; Cisplatin (.5 mg/kg) increased activity and frequency of spontaneous neuronal firing in wide dynamic range neurons</td>
<td>Cata 2008</td>
</tr>
<tr>
<td>Cytosine-arabinoside (2%)</td>
<td>Neurogenesis</td>
<td>i.c.v. for 14 days via osmotic pump</td>
<td>Male Sprague-Dawley rats</td>
<td>Cytosine-arabinoside suppresses neurogenesis and impaired learning/memory (fear conditioning)</td>
<td>Lau 2009</td>
</tr>
<tr>
<td>Vincristine</td>
<td>Morphology</td>
<td>Single i.c.v. injection in the dorsal hippocampus</td>
<td>Male Wistar rats (2 mo.)</td>
<td>Chemo had a dose dependant “all or none” effect on learning (MWM). Low dose had no effect, high dose group didn’t learn the task at all. Not a good drug for a learning/memory model</td>
<td>Eijkenboom 1999</td>
</tr>
<tr>
<td>Temozolomide (TMZ; 25 mg/kg)</td>
<td>Electrophysiology</td>
<td>I.P. inj. 3 consecutive days each week for 6 weeks</td>
<td>Male Sprague-Dawley rats (2 mo.)</td>
<td>TMZ reduced endogenous theta wave activity (associated with learning/memory), reduced neurogenesis, and impaired learning and memory (classical eye-blink task)</td>
<td>Nokia 2012</td>
</tr>
</tbody>
</table>

BCNU: Carmustine; GSH: Glutathione; GCEE: y-glutamyl cysteine ethyl ester; MDA: malondialdehyde; SOD: superoxide dismutase; MRP1: multidrug resistance-associated protein; TPI: triose phosphate isomerase; AST: aspartate aminotransferase; ALT: alanini amino-transferase; SOD: superoxide dismutase; PGE2: prostaglandin E2; ACH: acetylcholine.
Chapter 2: Methods

All procedures were approved by the Ohio State University and were conducted in accordance with National Institutes of Health guidelines for the Care and Use of Laboratory Animals (8th Edition) of animals and under protocols approved by the institutional animal care and use committee.

**Animals and environment**: Adult female BalbC (Charles River, Wilmington, MA) or C57BL6 (Charles River, Wilmington, MA) mice were housed in a temperature and humidity-controlled, ALAAC approved vivarium on a 14:10 light/dark cycle. All animals were allowed *ad libitum* access to food and water. All females were ovariectomized at least two weeks prior to being used in an experiment.

**Minocycline administration**: Minocycline was dissolved in the drinking water. The concentration of minocycline was adjusted on a daily basis to achieve a dose of approximately 90mg/kg/day. Mice received minocycline or the control water beginning two days prior to chemotherapy treatment, continuing through the end of the study. The minocycline and control water were prepared fresh daily and the total liquid consumed was recorded.
Chemotherapy administration: Animals received a single intravenous tail injection of either a combination of Doxorubicin (13.5mg/kg; TEVA Parenteral Medicines) and Cyclophosphamide (135 mg/kg; Baxter) or an equivalent volume of the vehicle (0.9% isotonic saline).

Intracerebroventricular Cannulation: A stereotaxic apparatus was used to implant a cannula into the left lateral ventricle of anesthetized mice (isoflurane) 2 days before chemotherapy treatment (cannula position: +0.02 posterior and – 0.95 lateral to bregma, extending 2.75 mm below the skull; Plastics One, Roanoke, Va). The cannula was connected by tubing to an Alzet minipump (Model 1002; Durect, Cupertino, Calif) that was implanted in the left lateral ventricle for constant infusion (0.25μL/day) of vehicle (artificial cerebrospinal fluid [aCSF]) or minocycline (10ug/day; <2μl) for 6 days. Tissue was then collected and analyzed for proper cannulation placement.

Behavioral Tests:
Locomotor Activity: Overall locomotor activity was tested using the open field, a 40 x 40 cm clear acrylic chamber lined with clean corncob bedding, inside a ventilated cabinet (Med Associates, St Albans, VT, USA). A frame at the base of the chamber consisting of 32 photobeams in a 16 x 16 arrangement, in addition to a row of beams above, detected the location of horizontal movements and rearing, respectively (Open Field Photobeam Activity System, San Diego
Instruments, San Diego, CA, USA). Total movement was tracked for 30 min and analyzed for the following: (1) the percentage of beam breaks in the center (inside 30 x 30 cm) of the open field, (2) number of rears and (3) total locomotor activity.

**Elevated Plus Maze:** Anxiety-like behavior was assessed in the elevated plus maze, which is a plus-shaped apparatus, elevated above the floor with two dark enclosed arms and two open arms. Each mouse was placed in the center of the test apparatus to begin and behavior was recorded on video for 5 min. An uninformed observer later scored videotapes for the number of entries and time spent in both the open and closed arms of the maze using Observer software.

**Forced Swim test:** To assess depressive-like behavioral responses in the forced swim test (Porsolt, Le Pichon & Jalfre, 1977), mice were placed in a clear 4 liter container filled with room-temperature water (22 ± 1°C) for 3 min. Behavior was recorded on video and subsequently scored with Observer software (Noldus, Wageningen, Netherlands) by an observer unaware of assignment to experimental groups. The behaviors scored were: (1) swimming or (2) floating/immobility (i.e., minimal movement necessary to keep head elevated above water surface).
**Learning and memory:** Learning and memory was assessed in the Barnes maze, a white circular polypropylene disk 91 cm in diameter (San Diego Instruments, CA), elevated 1.2 meters above the floor. The disk has 18 holes, 5 cm in diameter, evenly spaced around the perimeter. One of the holes leads to a dark escape box. Noldus tracking software (Leesburg, VA) records latency to reach the escape hole, path length, and number of errors. The testing occurs in a brightly lit room between one and six hours after the onset of the light phase in the colony. Mice received six days of training, consisting of three trials per day; the intertribal interval was approximately 10 min. If the mouse did not find the hole within the first two minutes of a trial, the latency was recorded as 120 seconds and the mouse was guided to the escape hole. A probe trial (90 s) was conducted on Day 7, in which the escape box was removed. Percent of visits to the target hole and latency to first reach the target hole were recorded. Beginning 3 hours after the probe trial, mice received 3 days of reversal trials in which the escape box was rotated 180 degrees away from its location; the reversal training also consisted of three trials per day.

**Microglia enrichment:** Brain tissue was minced and homogenized as previously described (Henry, Huang, Wynne, & Godbout, 2009). Single cell suspensions were resuspended in 70% Pecoll to which 50% Percoll, 35% Percoll, and 0% Percoll were layered atop respectively to generate a density gradient during a 20
minute centrifugation. The interface between the 70% and 50% layers consisting of enriched microglia was removed, washed and used for subsequent studies.

**LPS Microglial Stimulation:** Enriched microglia were resuspended in BV2 media, plated in a poly-l-lysine coated 96 well plate at 100,000 cells in 200uL media per well and placed in an incubator (37°C w/ 5% CO2) for one hour. Following incubation each well received 10uL of saline (control) or 10uL 0.40 µg/ml LPS (Sigma, St. Louis, MO) dissolved in sterile saline for 18 h at 37°C and 5% CO₂. Supernatants were collected and stored at -80 C for multiplex cytokine analysis and microglial cells were collected for PCR analysis.

**Protein Extraction and Determination of Cytokine Concentrations:** Brains were removed and processed through a 70um filter to generate a single cell suspension. After the cells were pelleted by centrifugation, cells were disrupted in 300uL of RIPA buffer (containing 0.5% bovine serum albumin) followed by sonication for five 3 second pulses. The homogenate was again spun down and the supernatant was collected, aliquoted and stored at -80°C until assay. The protein concentration of each sample was measured using the Pierce BCA protein assay (Thermo Scientific, Rockford, IL). The concentrations of IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, interferon γ (IFN-γ), tumor necrosis factor α (TNF-α and granulocyte macrophage colony-stimulating factor (GM-CSF) were measured using the Mouse Cytokine Group 1 kit (Biorad kit # M60-000011J) according to
the manufacturer’s protocol. For cytokine concentrations in the blood, IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12(P40), IL-12(P70), IL-13, IL-17, eotaxin, granulocyte colony-stimulating factor (G-CSF), KC, MCP-1, MIPα, MIP-1β, RANTES, IFN-γ, TNF-α, and GM-CSF were measured using the Mouse Cytokine Group 1 Panel 23-plex kit (Biorad kit # M60-009RDPD). All samples, standards, and blanks were run in duplicate using a Bio-plex 200 system plate reader and Bio-plex manager 6.1 software (Hercules, CA).

**Real-time PCR for tissue:** Brains were removed and stored in RNA later for four days at 0 degrees Celsius. The hippocampus and prefrontal cortex were removed and total RNA was extracted from these regions using a homogenizer (Ultra-Turrax T8, IKA Works, Wilmington, NC) and an RNeasy Mini Kit (Qiagen, Valencia CA) according to manufacturer’s protocol. Extracted RNA was suspended in 30µL of RNase-free water and RNA concentration was determined by a spectrophotometer (NanoDrop ND-1000, Wilmington, DE). A TaqMan 18S rRNA primer and probe set (labeled with VIC dye: Applied Biosystems, Foster City, CA) were used as a control gene for relative quantification in addition to the primers and probes for the desired genes of interest (Applied Biosystems, Foster City, CA). Amplification was performed on an ABI 7000 Sequencing Detection System by using Taqman Universal PCR master mix. The universal two-step RT-PCR cycling conditions used were: 50°C for 2min, 95°C for 10min followed by 40 cycles of 95°C for 15sec and 60°C for 1min.
Real-Time PCR for cells: Total RNA was extracted from enriched microglia by using a sonicater and an RNeasy Micro Kit (Qiagen, Valencia, CA) according to manufacturer’s protocol. Extracted RNA was suspended in 15 μL of RNase-free water, and RNA concentration was determined by a spectrophotometer (NanoDrop ND-1000). cDNA was made and PCR run as described above.

Flow Cytometry for brain and spleen: Brain tissue was obtained immediately following euthanasia and passed through 70μM cell strainers to obtain single-cell suspensions. Red blood cells were lysed and remaining cells were washed and resuspended in stain wash buffer. Cell surface Fc receptors were blocked via incubation with anti-CD16/32 antibody (ebioscience, San Diego, CA) and then washed with stain wash buffer. All antibody incubations were performed on ice in the absence of light. In general, the cells were incubated with mixtures of specific concentrations of fluorochrome-labeled antibodies according to experimental design. Cells were fixed in 2% paraformaldehyde in PBS and flow cytometry data was acquired using a BD LSRII instrument and analyzed using TreeStar FlowJo software (Ashland, Or). For any given marker, all of the analysis gates were identical in size and position.

Flow Cytometry for microglia: Microglia were enriched as previously described and placed in 5 mL tubes followed by Fc receptor blocking and staining as
described above. Flow cytometry data were acquired using a BD LSRII instrument (San Jose, CA) and analyzed using TreeStar FlowJo software (Ashland, Or). For any given marker, all of the analysis gates were identical in size and position.

**Hippocampal morphological analyses:** Mice were euthanized between 2 and 6 hours after the onset of the light phase in the colony room; brains were removed and impregnated using the FD Rapid GolgiStain Kit (FD NeuroTechnologies, Ellicott City, MD, USA) according to the manufacturer’s instructions. Brains were sliced at 100 mm, thaw mounted onto gelatin-coated slides that were coded in order to remove observer bias, counterstained with cresyl violet (Sigma, St Louis, MO, USA), dehydrated and coverslipped. Brains were assessed for hippocampal cell morphology and spine density in three hippocampal subfields: dentate gyrus, CA1 and CA3. Sections were visualized using a Nikon E800 brightfield microscope (Nikon, Burlington, VT, USA) and traced using Neurolucida software (MicroBrightField, Burlington, VT, USA) at a magnification of 200x for neuronal morphology and 1000 x for spine density. Four to five representative neurons were selected per area, per animal for tracing. Selected neurons were fully impregnated and lacked truncated dendrites. Whole cell traces were analyzed using NeuroExplorer software for cell body size and perimeter and dendritic length (MicroBrightField). Sholl analyses were conducted separately for apical and basilar dendrites. From each of the five neurons selected per animal four
segments > 20 mm were selected in the apical and basilar areas, respectively, (except in the dentate gyrus where granule cells lack bidirectional projections). All spine segments selected were at least 50 mm distal to the cell body. Spine density (spines per 1 mm) was calculated for each trace and averaged per cell, per area and per animal.

**Immunohistochemistry:** Mice were anesthetized (pentobarbital) and perfused transcardially with 0.1 mol/L phosphate-buffered saline followed by 4% paraformaldehyde. Brains were removed, post-fixed for 4 h, and then cryoprotected in 30% sucrose in 0.2 mol/L phosphate-buffered saline. The brains were sectioned coronally at 40 μm on a freezing microtome and rinsed 3 times for 5 minutes each in 0.1 mol/L phosphate-buffered saline. The sections were then incubated for 40 hours at 4°C with the appropriate antibodies. After 3 five minute rinses, the tissue was incubated with secondary antibodies for 2 days at room temperature. The tissue was again rinsed 3 times and then mounted onto Super Frost Plus slides (Fisher, Hampton, NH, USA). All images were captured using a Zeiss LSM 510 confocal microscope.

**Chemotherapy concentration in brain and blood:** Liquid chromatography tandem mass spectrometry (LC-MS/MS) was used to determine the concentration of doxorubicin and cyclophosphamide agents in the brain and blood from five minutes to twenty-four hours after treatment. After blood and
brain were collected, 200μL plasma sample was prepared immediately from blood. A 100uL plasma aliquot for cytoxan/ phosphoramide mustard assay was treated with semicarbazide to prevent 4-hydroxy-cytoxan from converting to phosphoramide mustard, and the other 100uL plasma aliquot was transferred into a separated micro centrifuge tube for assay of doxorubicin. Brain extract sample for cytoxan/ phosphoramide mustard assay was prepared from 100mg brain tissue homogenized with 250μL PBS and 25μL 2M semicarbazide solution; for doxorubicin the same amount of brain tissue was homogenized in 250μL of 50% MeOH/ 50% H2O. All the procedures were carried out on ice, all solutions pre-chilled to 4°C, and all samples were snap frozen on dry ice and stored at -80°C.

Two bioanalytical methods were developed and validated for quantification of doxorubicin, cytoxan and its active metabolite phosphoramide mustard in mouse plasma and brain using liquid chromatography tandem mass spectrometry (LC-MS/MS), one for simultaneous determination of doxorubicin, the other for cytoxan and phosphoramide mustard. Briefly, the cytoxan and phosphoramide mustard were extracted from plasma and brain extract samples with solid phase extraction (SPE) using Waters Oasis MAX cartridges. Doxorubicin was recovered from plasma and brain extract samples by protein precipitation with methanol. A Thermo Dionex RSILC system consisted of degasser, ternary pump, column oven and autosampler was used for solvent and sample delivery. A TSQ Quantum Ultra EMR mass spectrometer equipped with
an electrospray ionization (ESI) source was used for mass analysis and
detection. Both methods used an Agilent ZORBAX Extended C18 column
(50×2.1mm I.D, 3.5μm particle size) coupled with an Agilent Extend C18 guard
cartridge (2.1mm×12.5mm, 5μm particle size) at 40°C with a total run time of
6.0min. The chromatographic separation was performed using two linear gradient
elution programs with mobile phases consisted of H₂O with 0.2% formic acid and
methanol with 0.2% formic acid at a flow rate of 300μL/min for both assays. The
two methods carried out the MS detection with selected reaction monitoring
under positive mode using transitions from the precursor ions to product ions at
m/z 544.118-397.02 for doxorubicin, m/z 261.02-140.07 for cytoxan and m/z
221.02>142.05 for phosphoramide mustard. These assays were developed and
conducted by the Pharmacoanalytic Shared Resource of the Comprehensive
Cancer Center, OSU.

**Data analysis:** Student’s t-tests were used to analyze OF, EPM, FST, PCR, flow
cytometry, neuronal morphology, and bioplex when comparing between two
experimental groups (chemotherapy versus vehicle). A one-way analysis of
variance (ANOVA) was used to analyze OF, EPM, FST, PCR, flow cytometry,
bioplex, and neuronal morphology when analyzing the effects of minocycline on
chemotherapy; LSD post-hoc was used to assess between group differences.
For Barnes maze analysis repeated-measures ANOVA were conducted with
drinking and drug as the with-in subject factors and session as the between
subject factor to analyze latency to find the target hole, error rate, and path length. Following a significant result on repeated measures ANOVAs, comparisons were made between groups using one-tailed LSD post-hoc tests based on a priori hypotheses. Differences were considered statistically significant if \( p \) values were less than 0.05. All error bars represent standard error of the mean (SEM). Statistical analyses were conducted with SPSS software (version 22).

Chapter 3: Chemotherapy and Affective Behavior

While findings from clinical studies clearly suggest that cancer is associated with increased anxiety and depression, relatively few studies have assessed the effects of chemotherapy on depression and anxiety. Only recently has it been suggested that BC patients treated with chemotherapy are at increased risk of depression and anxiety relative to BC patients who are treated with surgery, radiotherapy or endocrine therapy (Redeker, Lev, & Ruggiero, 2000; Reece, Chan, Herbert, Gralow, & Fann, 2013; Torres et al., 2013). Up to forty percent of BC patients treated with chemotherapy suffer from depression and 27% from anxiety (Hack et al.; So et al., 2010). Furthermore, some specific drug regimens may be associated with increased risk; for example, treatment with a doxorubicin based regimen was nearly three times as likely to result in
depressive symptoms as a chemotherapy regimen not containing doxorubicin or a non-chemotherapy treatment (Avis et al., 2012). Similarly, a longitudinal study reported an increase in depression when treated with doxorubicin/cyclophosphamide, but not other regimens (Reece, Chan, Herbert, Gralow, & Fann, 2013). These data are particularly concerning because doxorubicin/ cyclophosphamide (AC) is a commonly used and effective regimen for treating BC. In addition to the obvious concern regarding the negative effect of affective disorders on the quality of life for BC patients, depression also has been associated with fatigue, sleep disturbance, decreased survival, and increased tumor growth and recurrence rates (Falagas et al., 2007; Goodwin, Zhang, & Ostir, 2004; Khan, Amatya, Pallant, & Rajapaksa, 2012; Liu et al., 2009; Meyer, Sinnott, & Seed, 2003; Redeker, Lev, & Ruggiero, 2000). Furthermore, depression can interfere with medical compliance (Colleoni et al., 2000). An additional impediment to treating depression in BC survivors is that several commonly prescribed classes of antidepressants can interfere with the efficacy of ET (Kelly et al., 2010). Thus, treating depression and anxiety in BC patients is currently a challenge.

It is not known why doxorubicin/cyclophosphamide is more commonly associated with chemotherapy-associated depression than other regimens, although it may be related to the inflammatory properties of doxorubicin (Tangpong et al., 2006). Findings from pre-clinical studies suggest that doxorubicin increases TNFα, IL-1b, and IL-8 in rodent models (Aluise et al.,
2011; Kang, Chess-Williams, Anoopkumar-Dukie, & McDermott, 2013; Niiya et al., 2003; Tangpong et al., 2011) and in vitro using human lung carcinoma cells (Niiya et al., 2003). Furthermore, in a clinical study BC patients treated with doxorubicin, cyclophosphamide, and 5-fluorouracil had higher concentrations of IL-6 than patients treated with cyclophosphamide, methotrexate, and 5-fluorouracil (Janselsins et al., 2012).

Pro-inflammatory cytokines are associated with depression in otherwise healthy individuals, as those with major depressive disorder have increased serum concentrations of IL-1, IL-6, and TNF-α (Maes et al., 1997; Mikova, Yakimova, Bosmans, Kenis, & Maes, 2001; Miller, Maletic, & Raison, 2009). More specifically, BC studies also report an association between depression and increased serum concentrations of IL-6 and inflammatory mediator NF-κB (Jehn et al.; Musselman et al., 2001; Torres et al., 2013). However, as all of these studies are correlative, they do not assess causation. Numerous preclinical studies, using a wide range of models, report that upregulation of the immune system can induce sickness behavior and depressive-like behavior (Dantzer, O'Connor, Freund, Johnson, & Kelley, 2008; Neigh et al., 2009; Norman et al., 2010; O'Connor et al., 2009; Wynne, Henry, & Godbout, 2009). Furthermore, inhibiting the increase in inflammation also ameliorates the depressive-like and anxiety-like behavior (Norman et al., 2010). For example, peripheral nerve injury-induced inflammation (IL-1b) and depressive-like behavior after spared nerve injury are ameliorated with administration of IL-1 receptor antagonist (Norman et
al., 2010). In a cardiac arrest mouse model, minocycline reduced ischemia-related microglial activation and anxiety-like behavior (Neigh et al., 2009). Social defeat also increases IL-6 and TNF-α expression and induces anxiety-like behavior; however, social defeat appears to be mediated by IL-1 since social defeat does not increase microglial activation or anxiety-like behavior in IL-1 receptor type-1-deficient mice (Wohleb et al.). Administration of lipopolysaccharide (LPS) has also been used to activate the immune system and induces depressive-like behavior that is ameliorated by minocycline (O'Connor et al., 2009). Thus, these data provide strong evidence for a causal relationship between elevated proinflammatory cytokines in the brain and affective changes in rodents. Taken together with the clinical studies that demonstrate correlative associations between serum cytokines and depression in BC patients being treated with chemotherapy (Torres et al., 2013) and the proinflammatory nature of AC-based chemotherapy (Janelins et al., 2012; Tangpong et al., 2011), it is reasonable to hypothesize that chemotherapy-induced neuroinflammation underlies the development of depressive-like and anxiety-like behavior.

The goals of this study are 1) to determine if the affective consequences of chemotherapy can be recapitulated in mice, 2) to determine if chemotherapy produces neuroinflammation, and 3) to establish whether a causal relationship exists between chemotherapy-induced neuroinflammation and changes in affective behavior. If inflammation is part of the causal mechanism linking chemotherapy and depressive-like behavior, then this research could offer new
therapeutic targets for the treatment of depression in BC patients. Alleviating depression could not only improve the quality of life for breast cancer patients, but also potentially lead to better health outcomes.

*Experimental Design*

**Experiment 1.** Ten adult Balb/C female mice were ovariectomized two weeks before the experiment. Seven mice received a single tail vein injection of doxorubicin (13.5 mg/kg) and cyclophosphamide (135 mg/kg) chemotherapeutic agents (AC); five received saline (vehicle). Three days after treatment, brains were collected; a small section of prefrontal cortex was removed from alternating hemispheres for PCR analysis. The remaining tissue was homogenized and prepared for analysis of cytokines via a bioplex assay.

**Experiment 2.** Fifteen Balb/C female mice were ovariectomized two weeks before the experiment. Three days before chemotherapy treatment, females were separated and housed one mouse per cage for the remainder of the experiment. Eight mice received a single tail vein injection of AC; seven received vehicle. Three days later, following intravenous injection, behavioral testing was performed between one and six hours after the onset of the light phase in the colony room; mice were allowed to habituate to the test room for 30 min prior to testing. Tests were performed in the following order: (1) open field (2) elevated plus maze, (3) forced swim test; the order of testing reflects arrangement from
the least potentially intrusive to most intrusive test. The mice were returned to their cage for ten minutes between tests.

**Experiment 3.** Twelve C57BL/6 adult female mice were ovariectomized two weeks before the experiment. Seven mice received a single tail vein injection of AC; five received the vehicle. Three days after treatment, behavior was assessed between 06:00 and 10:00 using the open field, elevated plus maze, and forced swim task, in that order. After each test mice were placed back in their cage for ten minutes before beginning the next test. One hour after behavior, brains were collected and prepared for analysis using flow cytometry.

**Experiment 4.** Forty Balb/C two month old female mice were ovariectomized two weeks before the experiment. Mice were injected with AC in the tail vein. Brains and blood were collected at various time points after treatment (5 min., 10 min., 15 min., 30 min., 1, 1.5, 2, 4, 6, 8, or 24 hours) with four mice per group. Serum and brain were processed for LC-MS/MS to determine drug concentration in the blood and brain.

**Experiment 5.**

Twenty Balb/C adult female mice were ovariectomized two weeks before the experiment. Two days before receiving AC treatment or vehicle (tail vein), mice were implanted with a cannula in the right lateral ventricle, receiving aCSF or
minocycline (10ug/day at .25uL/hour) throughout the remainder of the experiment. Three days following AC treatment, behavior was assessed between 06:00 and 10:00 using the open field, elevated plus maze, and forced swim task, in that order. After each test mice were placed back in their cage for ten minutes before beginning the next test. One hour after behavior, brains were collected and prepared for PCR analysis of mRNA.

**Experiment 6.**

Forty Balb/C two month old female mice were ovariectomized two weeks before the experiment. Beginning two days before receiving AC treatment (tail vein) and remaining until the end of the study, mice were administered either minocycline (90 mg/kg) in their drinking water or the typical water; the water was changed every day for each animal. Three days following AC treatment, behavior was assessed between 06:00 and 10:00 using the open field, elevated plus maze, and forced swim task, in that order. After each test mice were placed back in their cage for ten minutes before beginning the next test. One hour after behavior, brains were collected and prepared for PCR analysis of mRNA.
Results

Experiment 1

Cytokine Concentration in the Brain

Chemotherapy treatment upregulated protein expression of several pro-inflammatory cytokines within the brain (Figure 3.1). Three days after injections, chemotherapy treated mice had higher concentrations of pro-inflammatory cytokines tumor-necrosis alpha (TNFα; t₈=3.5, p<.01), and chemokine (C-C motif) ligand four (CCL4; t₈=2.2, p<.05) in the brain relative to control mice. IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, interferon γ (IFN-γ), and granulocyte macrophage colony-stimulating factor (GM-CSF), other pro-inflammatory markers, as well as anti-inflammatory marker IL-10 were unaffected by chemotherapy (p>0.05).

Pro-inflammatory Gene Expression in the Prefrontal Cortex of the Brain

Chemotherapy upregulated gene expression of several pro-inflammatory cytokines (Figure 3.2). Relative gene expression of TNFα (Figure 3.5 A; t₆=2.1, p<.05) and IL-6 (Figure 3.5B; t₈=4.1, p<.01) were increased in chemotherapy treated mice as compared to those injected with the vehicle.

Experiment 2

Depressive-like Behavior

Chemotherapy increased the time engaged in immobility, or behavioral despair, in the forced swim test (Figure 3.3A). Specifically, relative to vehicle
treated controls, mice treated with a combination of doxorubicin and cyclophosphamide spent significantly more time floating than actively swimming ($t_{13}=2.4$, $p<.05$), indicative of increased behavioral despair. Chemotherapy did not have an effect on the number of floating bouts (Figure 3.3B; $t_{13}=0.9$, $p>.05$) or latency to the first float (Figure 3.3C; $t_{13}=1.4$, $p>.05$), suggesting that chemotherapy-induced lethargy was not a factor.

**Anxiety-like Behavior**

Mice treated with chemotherapy showed anxiogenic behavior (Figure 3.4 A) as they spent less time exploring the open arms of the elevated plus maze relative to controls ($t_{13}=2.7$, $p<.05$). However, there was no chemotherapy effect on locomotor activity (Figure 3.4 B) as there was no difference between groups in the total number of arms entered (closed and open combined; $t_{13}=1.1$, $p>.05$).

**Locomotor Activity**

Chemotherapy does not affect overall locomotor activity (Figure 3.5). Mice treated with chemotherapy showed no difference in the total number of beams crossed in the open field chamber, indicative of locomotor activity, relative to vehicle treated mice ($t_{13}=1.9$, $p>.05$).
Experiment 3

Depressive-like Behavior in C57bl/6 Mice

Chemotherapy increased the time engaged in immobility, or behavioral despair, in the forced swim test (Figure 3.6A). Specifically, relative to vehicle treated controls, mice treated with a combination of doxorubicin and cyclophosphamide spent significantly more time floating than actively swimming ($t_{10}=2.1$, $p<.05$), indicative of increased behavioral despair. Chemotherapy did not have an effect on the number of floating bouts (Figure 3.6AB; $t_{10}=0.41$, $p>.05$) or latency to the first float (Figure 3.6C; $t_{10}=0.7$, $p>.05$), suggesting that chemotherapy-induced lethargy was not involved.

Anxiety-like Behavior in C57bl/6 Mice

Mice treated with chemotherapy showed anxiogenic behavior (Figure 3.7A) as they spent less time exploring the open arms of the elevated plus maze relative to controls ($t_{10}=1.9$, $p<.05$). However, there was no chemotherapy effect on locomotor activity (Figure 3.7 B) as there was no difference between groups in the total number of arms entered (closed and open combined; $t_{10}=1.1$, $p>.05$).

Locomotor Activity in C57bl/6 Mice

Chemotherapy does not affect overall locomotor activity (Figure 3.8). Mice treated with chemotherapy showed no difference in the total number of beams
crossed in the open field chamber, indicative of locomotor activity, relative to vehicle treated mice \((t_{10}=1.2, p>.05)\).

**Protein Expression of Microglial Activation Markers**

Chemotherapy increased protein expression of microglial activation markers within the brain (Figure 3.9). Three days following a single treatment of chemotherapy C57bl/6 mice had more cd11b+ cells \((t_{10}=2.5, p<.05)\) and increased protein expression of microglial activation markers CD80 \((t_8=3.1, p<.05)\) and CD86 \((t_8=2.2, p<.05)\) compared to control mice. There was no difference in expression of MHCII \((t_9=0.9, p>.05)\).

**Experiment 4**

**Time course concentrations of doxorubicin and cyclophosphamide**

Cyclophosphamide and its metabolite, phosphamide, are present in both the blood (Figure 3.10 A) and brain (Figure 3.10 B) within five minutes of i.v. administration. However, two hours after injection, only trace amounts are present in the brain, none in the blood. Doxorubicin is present in the blood up to 24 hours after injection, but not detected in the brain at any time point.
Experiment 5

Depressive-like behavior after i.c.v. minocycline administration

Minocycline ameliorated chemotherapy-induced immobility, or behavioral despair, in the forced swim test (Figure 3.11A; F_{3,15}=7.3, p<.01). Post-hoc analysis revealed that mice receiving minocycline and chemotherapy spent less time floating than chemo treated mice receiving aCSF (vehicle), suggesting that minocycline ameliorates the depressive-like behavior induced by chemotherapy. The number of floating bouts differed (Figure 3.11B; F_{3,15}=6.7, p<.05) in that both chemo groups floated more times than the vehicle groups (P<.05). There was no difference between groups in latency to first float (Figure 3.11C; F_{3,15}=1.1, p>.05).

Anxiety-like behavior after i.c.v. minocycline administration

Minocycline ameliorated chemotherapy-induced suppression of anxiogenic-like behavior, in the elevated plus maze (Figure 3.12; F_{3,15}=10, p<.01). Post-hoc analysis revealed that mice receiving minocycline and chemotherapy spent more time in the open arms than chemo treated mice receiving aCSF (vehicle), suggesting that minocycline ameliorates the anxiety-like behavior induced by chemotherapy.
Locomotor Activity

Minocycline (i.c.v.) does not affect overall locomotor activity (Figure 3.13). There were no differences in locomotor activity in mice receiving chemotherapy and/or minocycline (Figure 3.14; $F_{3,14}=1.1, p>.05$).

Effects of minocycline on gene expression of pro-inflammatory markers after AC

Minocycline (i.c.v.) ameliorated chemotherapy-induced gene expression of pro-inflammatory cytokine IL-6 in the pre-frontal cortex (Figure 3.14; $F_{3,15}=9, p<.01$). Post-hoc analysis revealed that mice receiving minocycline and chemotherapy had reduced gene expression of pro-inflammatory marker IL-6 relative to mice receiving normal drinking water and chemotherapy ($p < .05$). There were no differences for IL-1b or TNF-α (data not shown).

Experiment 6

Depressive-like behavior after oral minocycline administration

Minocycline (oral administration) ameliorated chemotherapy-induced immobility, or behavioral despair, in the forced swim test (Figure 3.15A; $F_{3,35}=4.2, p<.05$). Post-hoc analysis revealed that mice receiving minocycline and chemotherapy spent less time floating than chemotherapy treated mice receiving aCSF (vehicle), suggesting that minocycline ameliorates the depressive-like behavior induced by chemotherapy. The number of floating bouts differed (Figure...
3.15B; F3,35 =4.1, p<.05) in that the chemotherapy water group had more floating bouts than the vehicle water and chemotherapy minocycline (p<.05), but not vehicle minocycline (P>.05) groups. The vehicle minocycline group also had more floating bouts than the chemotherapy minocycline (p<.05), but not vehicle water (p>.05) groups. Given there is no specific effect of chemotherapy or minocycline on number of floating bouts and the chemotherapy minocycline group performed better than both the chemo water and vehicle minocycline groups, these results suggest that the number of floating bouts may not be a good measure of depressive-like behavior in this model. There was no difference between groups in latency to first float (Figure 3.15C; F3,15 =1.1, p>.05).

**Locomotor Activity**

Minocycline (oral administration) does not affect overall locomotor activity (Figure 3.16). There were no differences in locomotor activity in mice receiving chemotherapy and/or minocycline (Figure 3.14; F3,14=1.1, p>.05).

**Effects of minocycline on gene expression of pro-inflammatory markers after AC**

Minocycline (oral administration) ameliorated chemotherapy-induced gene expression of pro-inflammatory cytokine IL-6 in the pre-frontal cortex (Figure 3.17; F3,15=9, p<.01). Post-hoc analysis revealed that mice receiving minocycline and chemotherapy had reduced gene expression of pro-inflammatory marker IL-
6 relative to mice receiving normal drinking water and chemotherapy (p < .05). There were no differences for IL-1b or TNF-α (data not shown).

**Discussion**

Clinical studies suggest that treatment with doxorubicin and cyclophosphamide are more likely to result in depression and anxiety than a regimen using other chemotherapeutic agents (Avis et al., 2012; Reece, Chan, Herbert, Gralow, & Fann, 2013). Thus, I hypothesized that non-tumor bearing mice treated with doxorubicin and cyclophosphamide would also show an increase in depressive-like and anxiety-like behavior relative to vehicle treated mice. Indeed, relative to control mice, chemotherapy treated mice spent more time floating in the forced swim test, indicative of depressive-like behavior. Additionally, chemotherapy treated mice spent less time in the open arms of the elevated plus maze, indicative of anxiety-like behavior. Both BalbC and C57bl/6 mice were tested in all three tasks and showed similar results, suggesting that chemotherapy affects both of these strains in a similar manner. Importantly, results from the open field test suggest that chemotherapy did not have an impact on overall locomotor activity, thus it is unlikely that chemotherapy-induced lethargy was a confounding factor in these tests.

As a few clinical studies have found that chemotherapy is associated with an increase in serum proinflammatory markers (S. Kesler et al., 2013; Torres et al., 2013), we hypothesized that treatment with concomitant doxorubicin and
cyclophosphamide would increase proinflammatory markers within the brain. Indeed, three days after a single dose of chemotherapy gene expression, pro-inflammatory cytokines TNF-α and IL-6 and protein concentrations of TNF-α and CCL4 were higher relative to vehicle treated mice. Changes in affective behavior have been shown to be induced by an increase in pro-inflammatory cytokines secreted by activated microglia (Dantzer, O’Connor, Freund, Johnson, & Kelley, 2008; Wynne, Henry, & Godbout, 2009). Thus, we hypothesized that chemotherapy treatment would result in an increase in microglial activation markers. Indeed, microglia from mice treated with doxorubicin and cyclophosphamide expressed higher levels of activation markers CD80 and CD86.

CCL-4, a chemokine mainly known to be secreted by macrophages in the periphery, is also expressed by microglia and astrocytes and is associated with microglial chemotaxis and propagation of a pro-inflammatory signal (El Khoury & Luster, 2008). Microglia can also use CCL-4 to enhance the recruitment of additional microglia (Rock et al., 2004). Thus, it may be involved in microglial recruitment or propagation of the chemotherapy-induced pro-inflammatory signal within the CNS. Further research is needed to fully understand the role of increased CCL-4 after chemotherapy.

To evaluate the causal relationship between elevated proinflammatory cytokines and altered affective behavior following chemotherapy, we administered minocycline beginning several days prior to chemotherapy.
treatment. Minocycline is a lipid soluble broad-spectrum tetracycline antibiotic, most commonly used to treat patients with acne and Lyme disease. It readily crosses the BBB and is known to suppress microglial activation and associated increase in pro-inflammatory cytokines within the CNS through blockade of NF-kappa B nuclear translocation (Plane, Shen, Pleasure, & Deng, 2010). Thus, it was hypothesized that minocycline would ameliorate chemotherapy-induced increase in microglial activation and proinflammatory cytokines, subsequently ameliorating chemotherapy-induced depressive-like and anxiety-like behavior. Indeed, minocycline administration did concurrently reduce chemotherapy-induced depressive-like behavior, anxiety-like behavior, and gene expression of IL-6. However, there was not an effect of minocycline on protein expression of pro-inflammatory cytokines or microglial activation markers in the brain (data not shown) three days after AC treatment. It may be that the primary effects of doxorubicin and cyclophosphamide on microglia and proinflammatory cytokines may subside before our analysis at 72 hours. Future research needs to assess the effects of AC on microglial activation and cytokine expression at earlier time points. Pre-clinical studies report an increase in TNFα three hours after treatment with doxorubicin (Tangpong et al., 2006). Furthermore, determining cell death within the brain at various time points, including 72 hours, will also assist in determining the effects on neurons that may have already occurred by 72 hours after treatment. It also would be beneficial to assess the effects of doxorubicin and cyclophosphamide individually to determine whether both drugs contribute
equally to the resulting neuroinflammation and behavioral deficits. The effect of minocycline on depressive-like behavior and IL-6 gene expression was similar for both oral and central administration of minocycline, further reinforcing the hypothesis that the effect of chemotherapy on behavior and inflammation is centrally mediated. Furthermore, oral administration of minocycline is readily available in clinical settings.

These data suggest that doxorubicin and cyclophosphamide may induce depressive-like and anxiety-like behavior. Furthermore this effect may be mediated by an increase in pro-inflammatory cytokines released from activated microglia. More importantly, minocycline ameliorates the depressive-like and anxiety-like effects of AC. This is relevant for clinical research as minocycline is an antibiotic already prescribed and used in clinical settings. Concomitant administration of minocycline and chemotherapeutic agents may ameliorate the effects on affective behavior seen in a subset of patients treated with chemotherapy.
Figure 3.1 Chemotherapy increases protein expression of TNFα and CCL4 in the brain 72 hours after treatment. Proinflammatory proteins markers tumor necrosis factor alpha (TNFα) and chemokine (C-C motif) ligand 4 (also known as macrophage inflammatory protein 1β) were measured in whole brain homogenates using bioplex. The data are presented as mean (+SEM); an asterisk (*) indicates a significant difference between groups at p<0.05.
Figure 3.2 Chemotherapy increases mRNA expression of TNFα and IL-6, but not IL-1β in the prefrontal cortex of the brain 72 hours after treatment. Gene expression of proinflammatory protein markers tumor necrosis factor alpha (TNFα; A), IL-6 (B), and IL-1β (C) were measured in the prefrontal cortex using RT-PCR. The data are presented as mean (±SEM); an asterisk (*) indicates a significant difference between groups at p<0.05.
Figure 3.3 Chemotherapy increases depressive-like behavior in BALBc mice 72 hours after treatment. Depressive-like behavior was assessed during a three minutes test using the forced swim task. The data are presented as mean (+SEM); an asterisk (*) indicates a significant difference between groups at p<0.05.
Figure 3.4 Chemotherapy increases anxiety-like behavior in BalbC mice without changing the locomotor activity 72 hours after treatment. Anxiety-like behavior was assessed in a five minute task using the elevated plus maze. The data are presented as mean (+SEM); an asterisk (*) indicates a significant difference between groups at p<0.05.
Figure 3.5 Chemotherapy does not affect locomotor activity in BALBc mice 72 hours after treatment. Locomotor activity was assessed in a 30 minute task using the open field test. The data are presented as mean (±SEM); an asterisk (*) indicates a significant difference between groups at p<0.05.
Figure 3.6 Chemotherapy increases depressive like behavior in C57bl/6 mice 72 hours after treatment. Depressive-like behavior was assessed using a three minute forced swim task. The data are presented as mean (+SEM); an asterisk (*) indicates a significant difference between groups at p<0.05.
Figure 3.7 Chemotherapy increases anxiety-like behavior of C57bl/6 mice without affecting locomotor activity 72 hours after treatment. Anxiety-like behavior was assessed during a five minute test using the elevated plus maze. The data are presented as mean (±SEM); an asterisk (*) indicates a significant difference between groups at p<0.05.
Figure 3.8 Chemotherapy (72 hours) does not affect locomotor activity in C57bl/6 mice 72 hours after treatment. Locomotor activity was assessed in a 30 minute task using the open field test. The data are presented as mean (+SEM); an asterisk (*) indicates a significant difference between groups at p<0.05.
Figure 3.9 Chemotherapy increases microglial activation markers within the brain 72 hours after treatment. Protein expression of microglial activation markers cd11b, cd80, and cd86 were assessed in whole brain homogenates using flow cytometry. The data are presented as mean (+SEM); an asterisk (*) indicates a significant difference between groups at p<0.05.
Figure 3.10 Expression of chemotherapeutic agents in the blood and brain at different time points after treatment. Concentrations of cyclophosphamide, doxorubicin, and phosphamide mustard metabolite were measured in the blood (A; nmol/liter) and whole brain homogenates (B; nmol/gram) using liquid chromatography tandem mass spectrometry (LC-MS/MS). The data are presented as mean (+SEM).
Figure 3.11 Minocycline (i.c.v.) ameliorates chemotherapy-induced depressive-like behavior 72 hours treatment. Depressive-like behavior was assessed using a three minute forced swim task. The data are presented as mean (+SEM); groups containing the same letter are not significantly different from one another (p>0.05).
Figure 3.12 Minocycline (i.c.v.) ameliorates chemotherapy-induced anxiety-like behavior 72 hours after treatment. Anxiety-like behavior was assessed from a five minute task using the elevated plus maze. The data are presented as mean (+SEM); groups containing the same letter are not significantly different from one another (p>0.05).
Figure 3.13 Minocycline (i.c.v.) does not affect overall locomotor activity after 72 hour chemotherapy treatment. Locomotor activity was assessed from a 30 minute task using the open field test. The data are presented as mean (±SEM); groups containing the same letter are not significantly different from one another (p>0.05).
Figure 3.14 Minocycline (i.c.v.) ameliorates chemotherapy-induced gene expression of pro-inflammatory cytokine IL-6 in the prefrontal cortex 72 hours after treatment. Gene expression of proinflammatory marker IL-6 was measured in the pre-frontal cortex using RT-PCR. The data are presented as mean (+SEM); groups containing the same letter are not significantly different from one another (p>0.05).
Figure 3.15 Minocycline (oral) ameliorates chemotherapy-induced depressive-like behavior 72 hours treatment. Depressive-like behavior was assessed using a three minute forced swim task. The data are presented as mean (+SEM); groups containing the same letter are not significantly different from one another (p>0.05).
Figure 3.16 Minocycline (oral) does not affect overall locomotor activity after 72 hour chemotherapy treatment. Locomotor activity was assessed from a 30 minute task using the open field test. The data are presented as mean (+SEM); groups containing the same letter are not significantly different from one another (p>0.05).
Figure 3.17 Minocycline (oral) ameliorates chemotherapy-induced gene expression of pro-inflammatory cytokine IL-6 in the prefrontal cortex 72 hours after treatment. Gene expression of proinflammatory marker IL-6 was measured in the pre-frontal cortex using RT-PCR. The data are presented as mean (±SEM); groups containing the same letter are not significantly different from one another (p>0.05).
Chapter 4

The Effects of Chemotherapy on Cognition

The effects of chemotherapy on cognition have garnered much interest and more than a thousand papers on this topic have been published, many in breast cancer patients due to the relatively high survival rate. Chemotherapy-related cognitive impairment is commonly reported by BC patients (Myers, 2012). The majority of BC studies that incorporate cognitive self-report measures indicate that women who have been treated with chemotherapy report greater cognitive deficits than BC patients who have not been treated with chemotherapy and matched healthy controls. Longitudinal studies using tests to assess cognitive changes after chemotherapy report significant deficits following treatment. Furthermore, these deficits can be detected up to fifteen years after receiving chemotherapy (Yamada, Denburg, Beglinger, & Schultz, 2010).

Although the clinical studies in breast cancer patients provide convincing evidence that a significant proportion of women undergoing chemotherapy develop persistent cognitive and affective side effects, and despite the vast literature, the mechanism is unknown. Animal models have been beneficial in that they allow a systematic and controlled study of the physiological changes in the brain associated with treatment of individual or combined chemotherapeutic agents. As discussed previously, a majority of animal research has shown that
chemotherapeutic agents do indeed induce deleterious cognitive effects in non-tumor models (ElBeltagy et al., 2010; Konat, Kraszpulski, James, Zhang, & Abraham, 2008; Y. Li, V. Vijayanathan, M. E. Gulinello, & P. D. Cole, 2010; Mondie, Vandergrift, Wilson, Gulinello, & Weber, 2010; Reiriz et al., 2006; Winocur et al., 2012; M. Yang et al., 2010). In rodent models, cyclophosphamide impairs passive avoidance task learning, novel object recognition, and inhibitory avoidance in mice (Reiriz et al., 2006; M. Yang et al., 2010) while doxorubicin impairs inhibitory avoidance in rats (Liedke et al., 2009). Combined, these drugs impair passive avoidance and contextual fear (Konat, Kraszpulski, James, Zhang, & Abraham, 2008).

In the past decade, a growing number of pre-clinical studies have proposed potential mechanisms associated with chemotherapeutic neurotoxicity within the CNS, but no interventional studies have attempted to establish causation. The goals of the present study are 1) to determine the cognitive effects of AC treatment using the Barnes maze, 2) to assess the morphological effects of AC on neurons and 3) to determine whether minocycline is able to reduce the chemotherapy-induced cognitive deficits. If minocycline is able to reverse the cognitive deficits, then this research could offer the first treatment of cognitive deficits in BC patients.
Experimental Design

Experiment 1

Sixteen Balb/C female mice were ovariectomized two weeks before the experiment. Eight mice received a single tail vein injection of doxorubicin (13.5 mg/kg) and cyclophosphamide (135 mg/kg) chemotherapeutic agents (AC); eight received saline (vehicle). Cognitive testing using the Barnes maze began two days after treatment. After each test mice were placed back in their cage for ten minutes before beginning the next test. Following the last day of testing brains were collected and prepped for PCR analysis. Although behavior was performed for six days, statistical analysis for behavior was performed on the first five days as both groups learned the task by the 5th day.

Experiment 2

Twenty Balb/C female mice were ovariectomized two weeks before the experiment. Ten mice received a single tail vein injection of AC; ten received vehicle. Brains were collected six days after treatment and placed in Golgi Cox solution (FD Rapid Golgi Stain Kit) then sectioned (100 μm) for neuronal morphology analysis.
Experiment 3

Forty Balb/C female mice were ovariectomized two weeks before the experiment. Beginning two days before receiving AC treatment (tail vein) and remaining until the end of the study, mice were administered either minocycline (90 mg/kg) in their drinking water or the typical water; the water was changed every day for each animal. Cognitive testing using the Barnes maze began two days after treatment. Following the last day of testing, brains were collected and prepped for PCR analysis.

Results

Experiment 1

Learning and memory

Chemotherapy impaired spatial learning in the Barnes maze; both chemotherapy and vehicle-treated mice learned the maze as indicated by a significant decrease in latency to reach the target box ($F_{4,52}=67.3$, $p<.05$; Figure 4.1A) and number of errors ($F_{4,44}=3.3$, $p<.05$; Figure 4.1B) over the course of training. However, chemotherapy treated mice exhibited significantly longer latencies to reach the escape box than vehicle mice ($F_{4,52}=2.96$, $p<.05$). Likewise, chemotherapy treated mice exhibited significantly longer latencies to reach the escape box than pair housed mice on days 2, 3, and 4. ($t_{13}=2.3, 2.9, \text{ and } 2.5, \text{ respectively}$).
Cytokine gene expression

Chemotherapy upregulated gene expression of pro-inflammatory cytokines in the hippocampus (Figure 4.2) and prefrontal cortex (Figure 4.3) of mice after completion of the Barnes maze. In the hippocampus, relative gene expression was greater in the chemotherapy treated mice for IL-1β (Figure 4.3A; \( t_{11}=3.3, \ p<.05 \)) and IL-6 (Figure 4.3B; \( t_{11}=4.5, \ p<.01 \)) and approached significance for TNFα (Figure 4.3C; \( t_{11}=1.6, \ p=.07 \)). Chemotherapy also reduced prefrontal cortex gene expression of TNFα (Figure 4.4A; \( t_{10}=2.8, \ p<.05 \)) and approached significance for IL-1β (Figure 4.4B; \( t_{9}=1.4, \ p=0.1 \)).

Experiment 2

Neuronal morphology

Golgi Cox stain was used to assess neuronal dendritic spine density in the hippocampus as learning and memory is associated with an increase in spine density. Chemotherapy treatment reduced dendritic spine density in both apical and basal dendrites of the CA1 and CA3 (Figure 4.4; apical CA1: \( t_{18} = 2.76; \ p<.05 \); basal CA1: \( t_{18} = 3.27; \ p<.01 \); apical CA3: \( t_{17} = 3.18; \ p<.01 \); basal CA3: \( t_{17} = 3.8; \ p<.01 \)) as well as the basal dendrites of the dentate gyrus in the hippocampus (\( t_{18} = 2.78; \ p<.05 \)). There was no effect of chemotherapy on the apical dendrites in the dentate gyrus (\( t_{18} = 2.78; \ p>.05 \)).
Experiment 3
Learning and memory

Minocycline (90 mg/kg/day) ameliorated chemotherapy-induced cognitive impairment in the Barnes maze (Figure 4.5) Repeated measures analysis reported differences between groups (F_{4,112}=3.6, p<.05) with post-hoc analysis revealing that chemotherapy treated mice receiving water exhibited significantly longer latencies to reach the escape box than all other groups (p < .05), including the group of chemo mice receiving minocycline, which did not differ from either of the non-chemo treated groups. Likewise, chemotherapy treated mice had significantly more errors on day 3 (F_{3,31}=6.6, p<.05) and traveled longer distances on days 3 (F_{3,28}=7.9, p<.01;) and 4 (F_{3,28}=5.1, p<.01) than the other three groups although the overall repeated measures analyses were not significantly different (errors: F_{4,112}=0.99, p>.05; distance: F_{4,112}=1.15, p>.05).

Gene expression of pro-inflammatory markers

Minocycline (90 mg/kg/day) ameliorated chemotherapy-induced gene expression of pro-inflammatory cytokine IL-6 in the prefrontal cortex (Figure 4.6A; F_{3,26}=4.6, p<.05). Post-hoc analysis revealed that mice receiving minocycline and chemotherapy had reduced gene expression of pro-inflammatory marker IL-6 relative to mice receiving normal drinking water and chemotherapy (p < .05). The data approached significance for IL-6 in the hippocampus (Figure 4.6B; F_{3,24}=1.76, p=.09) For IL-1β receptor in the prefrontal cortex there was an overall
difference (Figure 4.6C; $F_{3,27} = 3.69, p<.05$) with post-hoc analysis revealing higher IL-1β in the water+chemotherapy group than the water+vehicle and minocycline+vehicle groups and approached significance in comparison to minocycline chemo group ($p=.06$). There were no differences for IL-1β or TNF-α (data not shown).

**Discussion**

Clinical findings suggest that breast cancer patients treated with chemotherapeutic agents are more likely to experience cognitive deficits than non-treated controls (Ahles et al., 2010; Collins, Mackenzie, Stewart, Bielajew, & Verma, 2009). Furthermore, one study found that patients receiving AC-based treatment reported more cognitive deficits than those receiving other chemotherapeutic agents (Janelinsins et al., 2012). Thus, I hypothesized that non-tumor bearing mice treated with doxorubicin and cyclophosphamide would also show an increase in cognitive deficits relative to vehicle treated mice. Indeed, relative to control mice, chemotherapy treated mice showed reduced learning and memory ability in the Barnes maze in that they spent more time, committed more errors, and traveled a greater distance before finding the escape box than the vehicle-treated mice. Similar to our previous findings, we hypothesized that treatment with concomitant doxorubicin and cyclophosphamide would increase proinflammatory markers within the brain. Indeed, gene expression of pro-
inflammatory cytokines IL-6 and IL-1β in the hippocampus and TNF-α in the prefrontal cortex were elevated in AC treated mice relative to control mice. As dendritic spine density on neurons within the hippocampus is frequently associated with learning and memory (Leuner, Falduto, & Shors, 2003; Moser, Trommald, & Andersen, 1994), it was hypothesized that AC treatment would result in a decrease in dendritic spine density within the hippocampus. Indeed, mice treated with chemotherapy had fewer dendritic spines within the CA1, CA3, and dentate gyrus of the hippocampus than control mice. As previously discussed, minocycline has an anti-inflammatory effect within the CNS and was successful in ameliorating chemotherapy-induced depressive-like and anxiety-like behavior. Thus, it was hypothesized that minocycline would ameliorate chemotherapy-induced increase in proinflammatory cytokines, subsequently ameliorating the associated cognitive deficits. Indeed, minocycline administration did ameliorate chemotherapy-induced increase in IL-6 gene expression in the prefrontal cortex and approached significance in ameliorating IL-6 increase in the hippocampus and IL-1β receptor in the prefrontal cortex. As increased IL-1β receptor expression enhances the effects of IL-1β, suppressing the expression of IL-1β receptor reduces the downstream effects initiated by IL-1β binding to its receptor, thus dampening the overall effect of IL-1β. Minocycline also ameliorated chemotherapy-induced learning and memory deficits in the Barnes maze. There was no minocycline effect on IL-1β or TNF-α. As clinical studies have reported a reduction in gray matter after chemotherapy treatment (de Ruiter
et al., 2011; Inagaki et al., 2007), and pre-clinical studies have reported reduction in neurogenesis (ElBeltagy et al., 2010; Janelins et al., 2010), future studies need to assess the effects of AC on cell death and neurogenesis, as minocycline may affect one or both of these processes.

These data provide evidence in support of the hypothesis that doxorubicin and cyclophosphamide induce deficits in learning and memory. Furthermore this effect is mediated by an increase in pro-inflammatory cytokines. Most importantly, minocycline ameliorates the chemotherapy-induced deficits in learning and memory. This is relevant for clinical research as currently there is no treatment for cognitive deficits in cancer patients and many breast cancer patients experience “chemo-fog”. Concomitant administration of minocycline and chemotherapeutic agents may ameliorate the effects on cognitive ability reported in a subset of patients treated with chemotherapeutic agents. As minocycline is an antibiotic already prescribed and used in clinical settings, a clinical trial should be relatively easy to begin and is needed to test the effect of minocycline in breast cancer patients getting ready to undergo chemotherapy treatment. If minocycline is able to prevent the onset of cognitive deficits with chemotherapy, then this research could offer the first treatment of cognitive deficits in BC patients.
Figure 4.1 Chemotherapy impairs learning and memory in the Barnes maze 2-7 days after treatment. Learning and memory was assessed using latency to the hole and number of errors over six days using the Barnes maze; an asterisk (*) indicates a significant difference between groups at p<0.05.
Chemotherapy increases mRNA expression of IL-1b and IL-6 in the hippocampus 7 days hours after treatment. Gene expression of proinflammatory protein markers IL-1β (A), IL-6 (B), and TNFα (C), were measured in the hippocampus using RT-PCR. The data are presented as mean (+SEM); an asterisk (*) indicates a significant difference between groups at p<0.05.
Figure 4.3 Chemotherapy increases mRNA expression of TNFα in the prefrontal cortex 7 days after treatment. Gene expression of proinflammatory protein markers TNFα (A) and and IL-1β (B), were measured in the prefrontal cortex using RT-PCR. The data are presented as mean (+SEM); an asterisk (*) indicates a significant difference between groups at p<0.05.
Figure 4.4 Chemotherapy decreases dendritic spine density in the hippocampus 3 days after treatment. Dendritic spine density was quantified in the CA1, CA3, and dentate gyrus regions of the hippocampus following golgi-cox staining. The data are presented as mean (±SEM); an asterisk (*) indicates a significant difference between groups at p<0.05.
Figure 4.5 Minocycline ameliorates chemotherapy-induced cognitive impairment in the Barnes maze 2-6 days after treatment. Learning and memory was assessed using latency to the hole, number of errors, and distance to the hole over six days using the Barnes maze; an asterisk (*) indicates a significant difference between groups at p<0.05.
Figure 4.6

Figure 3.17 Minocycline ameliorates chemotherapy-induced gene expression of pro-inflammatory cytokine IL-6 in the prefrontal cortex 6 days after treatment. Gene expression of proinflammatory marker IL-6 and IL-1β were measured in the pre-frontal cortex (A and C) and hippocampus (B) using RT-PCR. The data are presented as mean (+SEM); groups containing the same letter are not significantly different from one another (p>0.05).
Chapter 5

Conclusion

Breast cancer is the most frequently diagnosed cancer in women; one in eight women in the U.S. will be diagnosed with this disease during their lifetime (Howlader N, 2011). Due to improved detection and treatment advances, the overall 5-year and 10-year survival rates for breast cancer are now 90% and 84.5% respectively (Howlader N, 2011). Thus, there is a growing population of breast cancer survivors for whom persistent side effects of treatment could impinge upon quality of life. Three of the most common side effects reported in breast cancer patients receiving chemotherapy are cognitive deficits, depression, and anxiety. These symptoms affect quality of life and are associated with decreased survival, increased recurrence rates, and increased tumor growth in breast cancer patients, although it is not clear if a causal relationship exist between depression and these outcome measures (Falagas et al., 2007; Goodwin, Zhang, & Ostir, 2004; Khan, Amatya, Pallant, & Rajapaksa, 2012; Meyer, Sinnott, & Seed, 2003; Redeker, Lev, & Ruggiero, 2000).

Despite the high incidence of cognitive and affective symptoms, the mechanisms through which chemotherapy may be affecting brain function remains a matter of speculation; prior to the research described in this thesis, no causal mechanism had been established in either humans or other animals. Furthermore, there are currently no standard pharmacological treatments for
chemotherapy-induced cognitive dysfunction and treatment of depression using conventional pharmaceutical methods is limited by potential interference with the effectiveness of endocrine therapy (Kelly et al., 2010). My studies not only demonstrate that both the cognitive and affective side effects of chemotherapy are related to neuroinflammation, but provide strong preclinical evidence that minocycline, an approved tetracycline analogue with good tolerability, can alleviate both of these side effects.

The goals of this dissertation were to assess the effects of doxorubicin and cyclophosphamide on cognitive and affective behavior, investigate a possible mechanism, and test the effects of minocycline on suppressing chemo-induced changes in behavior using a mouse model.

Based on both clinical and pre-clinical findings, it was hypothesized, in Chapter 3, that a single dose of AC would increase depressive-like and anxiety-like behavior. Indeed, a single treatment [75% human equivalent dose (HED)] of doxorubicin and cyclophosphamide increased depressive-like and anxiety-like behavior. Given the scarcity of the literature, both clinical and pre-clinical, associating depression and anxiety with chemotherapy, these findings are beneficial, specifically in a non-tumor model, suggesting that the chemotherapeutic agents may increase depressive and anxiogenic symptoms.

In Chapter 4, I tested the hypothesis that AC treatment would induce learning and memory deficits. Indeed, mice receiving a single dose of concomitant doxorubicin and cyclophosphamide (75% HED) showed impaired
cognitive ability in that they spent more time, had more errors, and traveled a greater distance before reaching the target hide box. Using a novel cognitive test for this field, the Barnes maze, these findings support findings from previous rodent models suggesting that chemotherapy may induce cognitive deficits in non-tumor bearing rodents (ElBeltagy et al., 2010; Reiriz et al., 2006; M. Yang et al., 2010).

Both clinical and pre-clinical studies have found doxorubicin to be associated with an increase in pro-inflammatory markers in both the blood and the brain (Aluise et al., 2011; Janelins et al., 2012; Kang, Chess-Williams, Anoopkumar-Dukie, & McDermott, 2013; Tangpong et al., 2011). Thus, it was hypothesized that treatment with AC would increase pro-inflammatory markers within the brain. Indeed, gene expression of IL-6, TNF-α, and IL-1b were higher in chemotherapy-treated mice relative to controls in the hippocampus and prefrontal cortex. Furthermore, levels of pro-inflammatory cytokines TNF-α and CCL4 were higher in chemotherapy-treated mice than controls.

Numerous pre-clinical studies, using a wide range of models, report that upregulation of microglia within the brain increases pro-inflammatory cytokines which can induce sickness response and depressive-like behavior (Dantzer, O'Connor, Freund, Johnson, & Kelley, 2008; Neigh et al., 2009; Norman et al., 2010; O'Connor et al., 2009; Wynne, Henry, & Godbout, 2009). Furthermore, inhibiting the increase in inflammation also ameliorates the depressive-like and anxiety-like behavior (Norman et al., 2010). Clinical breast cancer studies also
report an association between depression and increased serum concentrations of IL-6 and inflammatory mediator NF-κB (Jehn et al.; Musselman et al., 2001; Torres et al., 2013). Thus, it was hypothesized that microglia mediate the effects of chemotherapy on depressive-like and anxiety-like behavior. Although we did detect an increase in microglial activation markers CD80 and CD86 three days after treatment, more extensive studies on enriched microglia, with and without LPS stimulation, showed no chemotherapy effect on microglial activation. To better understand the potential role that microglia may have in mediating chemotherapy and behavior, future studies will need to assess microglial activation at earlier time points, as it is possible that microglia do become activated after chemotherapy treatment, but then return to a quiescent state within 72 hours after treatment. It is also possible that astrocytes may play a role in mediating these effects, which needs to be addressed in future studies.

Minocycline is a lipid soluble broad-spectrum tetracycline antibiotic that readily crosses the BBB and is known to suppress microglial activation and associated increase in pro-inflammatory cytokines within the CNS through blockade of NF-kappa B nuclear translocation (Plane, Shen, Pleasure, & Deng, 2010). Thus, it was hypothesized that minocycline would ameliorate chemotherapy-induced increase in microglial activation and proinflammatory cytokines, subsequently ameliorating chemotherapy-induced depressive-like and anxiety-like behavior. Indeed, minocycline administration did ameliorate chemotherapy-induced depressive-like behavior, anxiety-like behavior, and gene
expression of IL-6. However, there was not an effect of minocycline on protein expression of pro-inflammatory cytokines or microglial activation markers in the brain. Further research is needed to understand the role of microglia and inflammation in mediating the effects of chemotherapy on behavior. Specifically, kinetic studies would be beneficial in assessing the time course of inflammation from the periphery to the brain, followed by the cell-specific effects within the brain.

Although the mechanism is not fully understood, the main purpose of this proposal was to assess the possibility of minocycline being able to suppress or ameliorate the effects of AC on behavior. Administration of minocycline directly into the brain via intracerebral cannulation was effective in ameliorating the chemo-induced increases in depressive-like and anxiety-like behavior. Oral administration of minocycline was also effective at reversing the effects of chemotherapy on depressive-like behavior. There was not an effect of minocycline on anxiety-like behavior, however, when administered orally. It may be that a higher dose of minocycline is needed to have an effect on anxiety-like behavior; further research is needed to test this hypothesis. These findings are clinically significant as oral route is the easiest and most convenient mode of treatment administration and i.c.v. administration would not be very plausible in treating humans.

Minocycline was also successful in ameliorating the effects of chemotherapy on cognition. Mice receiving a single AC injection receiving normal
drinking water spent more time searching for the hide box and had more errors on day 3 and traveled a greater distance on days three and four than the other groups. Chemotherapy treated mice receiving minocycline in their drinking water performed significantly better on the Barnes maze than the non-minocycline chemotherapy group. Furthermore, the time spent searching for the hide box was almost identical on each test day for the minocycline treated chemo group as the two control groups, suggesting that minocycline may be able to completely abolish the cognitive deficits induced by chemotherapy. In is also important to note the minocycline itself did not have an effect on behavior, as noted by the similarity in behavior between the water vehicle group and the minocycline vehicle group.

As clinical studies have reported a reduction in gray matter after chemotherapy treatment (de Ruiter et al., 2011; Inagaki et al., 2007), and pre-clinical studies have reported reduction in neurogenesis (ElBeltagy et al., 2010; Janelinsins et al., 2010), future studies need to assess the effects of AC on cell death and neurogenesis, as minocycline may affect one or both of these. As breast cancer patients receive multiple doses of chemotherapeutic drugs, further pre-clinical research is also needed to determine if multiple treatments results in more severe cognitive and affective side effects.

My data suggest that a single administration of doxorubicin and cyclophosphamide is enough to alter cognitive and affective behavior. Furthermore, this effect is likely to be mediated by an increase in pro-
inflammatory cytokines released from activated microglia. Minocycline is a lipid soluble broad-spectrum tetracycline antibiotic, most commonly used to treat patients with acne and Lyme disease. It readily crosses the BBB and is known to suppress microglial activation and associated increase in pro-inflammatory cytokines within the CNS through blockade of NF-kappa B nuclear translocation (Plane, Shen, Pleasure, & Deng, 2010). My studies indicate that minocycline ameliorates the cognitive, depressive-like and anxiety-like effects of AC in rodents. This is relevant for clinical research as many breast cancer patients experience chemotherapy-related neuropsychological deficits and there are currently no standard pharmacological treatments for chemotherapy-induced cognitive dysfunction. Furthermore, treatment of depression using conventional pharmaceutical methods is limited by potential interference with the effectiveness of endocrine therapy (ET; Kelly et al., 2010). These side effects can compromise quality of life by amplifying the physical symptoms and functional impairments experienced by the women (Fann et al., 2008). In addition, untreated depression and concern about side effects can contribute to poor treatment adherence (Colleoni et al., 2000).

Concomitant administration of minocycline and chemotherapeutic agents may ameliorate the effects on cognitive ability seen in a subset of patients treated with chemotherapeutic agents. As minocycline is an antibiotic already prescribed and used in clinical settings, a clinical trial should be relatively easy to begin and is needed to test the effect of minocycline in breast cancer patients.
getting ready to undergo chemotherapy treatment. If minocycline is able to reverse the neuropsychological deficits in clinical trials, then this research could offer the first treatment of cognitive deficits in BC patients and be a safe option for treating chemo-induced depression and anxiety.
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