

Effects of chemotherapy on neural processes during cognitive functioning in early-stage breast cancer patients: An fMRI study

Nancy J. Wallis

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School of Psychology
Faculty of Social Sciences
University of Ottawa

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Abstract

Functional Magnetic Resonance Imaging (fMRI) was used to examine brain activity in women with early stage Breast Cancer (BC) and to compare their neural profiles to a matched control group. This was accomplished as participants performed two working memory tasks, before and at two time points following the chemotherapy intervention of the BC group. Nineteen BC patients between the ages of 18 and 65 years were recruited from the Ottawa Hospital Regional Cancer Centre. The nineteen control participants were matched on sex, language, age and education. The results, from whole brain analyses, show significant differences in neural activity between BC patients and matched control participants during both verbal and visuospatial working memory tasks, before and right after chemotherapy. However, these differences were no longer observed one year post chemotherapy for verbal WM processing. Performance results were not significantly different between groups until the third imaging sessions when patients made significantly more errors of omission than controls for both tasks. Importantly, mood, anxiety and fatigue all played significant roles in the observed findings demonstrating the multifaceted nature of the impact of both cancer and chemotherapy on neural function during working memory. This is one of the first fMRI studies to measure neural activations during cognitive performance both before and after chemotherapy in BC patients and a control group while controlling for many potentially confounding variables. While BC patients should be made aware of the potential cognitive challenges they might face before, during and shortly after treatment, they can also feel reassured that these impairments may not be long lasting.

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Dedication

I dedicate this thesis to my parents, Clayton & Audrey. You have loved me unconditionally throughout my life, and I thank you immensely for this. Without such a strong supportive foundation, I would not be where I am today.

Effects of chemotherapy on neural processes during cognitive functioning in early-stage breast cancer patients: An fMRI study

Breast Cancer (BC) is currently the most prevalent cancer amongst women.

Approximately 23,000 Canadian women are diagnosed with BC each year (Breast Cancer Society of Canada, 2013). This can be translated into an average of 64 women per day being diagnosed. Following surgery, it is common for BC patients to receive adjuvant chemotherapy to fully eradicate the cancer. Chemotherapy involves the administration of drugs systemically with the purpose of destroying the cancer cells, thereby increasing chances for long term survival. During this process normal healthy cells can be damaged resulting in a number of adverse side effects. Cognitive impairment is considered to be one such adverse effect.

Cognitive impairment, that is thought to result from chemotherapy intervention, is referred to as chemo brain, or the more popular term chemo fog. Due to the development of early BC detection methods, systemic chemotherapy regimens, and a subsequent rise in survival rates, this phenomenon has been both observed and investigated in BC patients over the past few decades. Many cancer survivors who have had toxic treatments like chemotherapy experience short-term memory loss and difficulty concentrating during and shortly after treatment (Ahles & Saykin, 2000; Castellon, Silverman & Ganz, 2005; Meyers, 2000). Indeed, BC survivors often self-report negative changes in memory, concentration, and other cognitive abilities following chemotherapy treatment. There are no guidelines with specific criteria to diagnose a cognitive impairment in this population. According to the literature, the cognitive domains most commonly affected in BC survivors include working memory, executive functioning and processing speed (Shilling et al., 2005; Steward, et al., 2007; Collins et al., 2012; Ahles, T., 2012). Despite the fact that investigations into chemo fog have been going on for years, the extent of chemo fog in BC

patients remains uncertain, and the neural mechanisms responsible for these problems are not fully understood.

Neuropsychology Findings

Duration of Impairment

The accumulated body of neuropsychological research on chemo fog is varied, but has contributed significantly to our understanding of chemo fog. The literature differs in its findings regarding the duration of cognitive impairment found in BC patients, including the possibility of both temporary and long term effects. For example, Brezden and colleagues (2000) published results that showed cognitive impairment for a group of women receiving adjuvant chemotherapy, compared to a group of healthy controls, when assessing six domains of cognitive functioning. Of the six domains (memory, language, visual-motor, spatial, attention/concentration, and self-regulation/planning), impairment was observed in memory and language only. Differences between groups remained significant even when age, education level and menopausal status were controlled for. However, it is important to note that there was a third group of participants in the Brezden study, women who had completed adjuvant chemotherapy at least one year prior to the start of the study (average time since completion of chemotherapy for this group was two years). No significant differences were reported between this last group and either of the other two groups in terms of cognitive functioning. These results suggest short-term impairment only- i.e., during the course of treatment or shortly thereafter. Collins et al. (2009) observed similar findings investigating the impact of chemotherapy on cognitive functioning in BC patients. Comprehensive neuropsychological test results were compared before chemotherapy, one month after chemotherapy, and again one year later between a BC group who had received a standard dose adjuvant chemotherapy with or without hormonal treatment, to

another BC group receiving adjuvant hormonal therapy only. Findings indicated that one-third of the women receiving a standard dose of adjuvant chemotherapy experience subtle disturbances in cognition, particularly in the working memory domain, during and shortly following treatment compared to a control group of women receiving adjuvant hormonal therapy only. Differences were no longer observed one year later. Still, others have found impairment to be longer. Schagen et al. (1999, 2002) reported that cognitive impairments were observed at two years post chemotherapy in BC survivors (but no longer present at four years post-treatment), and Ganz et al. (2002) reported findings of cognitive decline in BC patients five to ten years after initial diagnosis.

Dose Dependent Hypothesis

Variability in the length of impairment as well as the intensity for BC survivors may be explained in part by other factors such as the dose of chemotherapy administered. Van Dam and colleagues (1998) reported that a high dose chemotherapy regime in BC patients resulted in higher cognitive impairment compared to those receiving a standard dose. The sample was made up of 34 BC patients treated with a high dose chemotherapy regime plus tamoxifen (a drug commonly used with BC patients to block the binding of estrogen to the receptors of certain BC cells which can result in the growth of a malignant tumour), 36 BC patients treated with a standard dose chemotherapy regime plus tamoxifen, and 34 *control* Stage I BC patients with local therapy only (i.e., surgery or radiation- excluding CNS radiation). BC patients treated with high-dose chemotherapy appeared to have an 8.2-times higher risk of having cognitive impairment in comparison with the *control* BC patients, and a 3.5 times higher risk compared to the BC group who received a standard dose of chemotherapy. However, the inclusion of the

tamoxifen protocol may have had an impact on findings. Discussion of adjuvant therapies will follow.

Ahles et al. (2002) compared the neuropsychological functioning of long-term BC survivors (five years post-diagnosis and cancer free at the time of testing) who had been treated by standard dose systemic chemotherapy to those who had local therapy (surgery or radiation therapy) only. Significant differences were found in the domains of verbal memory and psychomotor functioning with the chemotherapy group performing more poorly. Moreover, even though most (85%) participants in this study received the same type of chemotherapy regimen (one standard dose), it was reported that the more cycles of chemotherapy undergone within a regime, the lower the neuropsychological performance. Recently, others have found the number of chemotherapy cycles appears to be a risk factor for cognitive dysfunction in women with BC (Wefel et al., 2010).

Impact of Adjuvant Therapies

It is likely that other types of adjuvant therapies also contribute to cognitive changes. For example, Bender et al. (2005) conducted a prospective study exploring multiple domains of cognitive functioning in women with Stage I or Stage II BC with and without Tamoxifen. Participants in the first group received chemotherapy, but were diagnosed as negative for hormone receptor BC, and thus did not receive tamoxifen. However, participants in the second group had hormone receptor positive BC resulting in treatment that included both chemotherapy and tamoxifen. Lastly, a control group comprising BC patients who had received no chemotherapy or tamoxifen intervention was recruited. All participants were asked to perform a battery of neuropsychological tests at three different time points assessing attention, learning and memory, psychomotor speed, mental flexibility, visuo-constructional abilities, executive

functioning and general intelligence. Measurements were administered as follows: 1) after surgery and prior to the initiation of adjuvant therapy in groups 1 and 2 and post surgery for group 3; 2) within one week post chemotherapy for groups 1 & 2 and a comparable timeline for group 3; and 3) followed by a final assessment one year post time two for all three groups.

Results indicated that the women who had received chemotherapy showed impairment on measures of memory one year post chemotherapy, whereas the control group did not show any deterioration. However, the women who received chemotherapy plus tamoxifen exhibited the most memory impairment (specifically visual memory and verbal working memory).

Interestingly, these results are in contrast to the findings of Paganinini-Hill and Clark (2002) which tamoxifen was not found to have an effect on cognitive functioning in women with BC. Study design concerns including the lack of a pre chemotherapy baseline measure in the 2002 study were indicated by Bender et al as the most likely explanation for the observed differences. Collins et al. (2009) reported similar findings showing that BC patients taking tamoxifen, or another form of hormonal therapy, anastrozole, were more likely than healthy controls to show consistent cognitive decline. Testing was administered around the initial time of treatment and approximately six months later with processing speed and verbal memory being most affected.

Impact of Menopause

Other researchers have looked more closely at the role of instant menopause on cognition, particularly investigating hormonal replacement therapy as yet another potential factor to consider in BC chemo fog research. It is important to note that at age 40 approximately 40% of women will experience permanent cessation of menses post chemotherapy, and as patient age increases, so does the risk of instant menopause (Goodwin et al., 1999). Chemotherapy for BC dramatically reduces estrogen levels which can induce menopause. As a result, many BC patients

undergo hormonal replacement therapy to compensate for lack of hormone production. The impact of hormonal replacement therapy on cognitive function is ambiguous. A number of studies suggest that hormonal replacement therapy might enhance verbal memory performance and decrease the risk for developing dementia in postmenopausal women (Maki et al., 2001; Miller et al., 2001; Sherwin, 2003.) Other studies report that hormonal replacement therapy impairs memory performance for women, and increases the risk for developing dementia (Rapp et al., 2003; Resnick et al., 2006; Shumaker et al., 2003, 2004). Specific to BC survivors, Ahles and colleagues (2002) reported no significant cognitive differences between women who received chemotherapy and endocrine treatment compared to those who only received chemotherapy, whereas Castellon et al. (2004) found greater cognitive decline in BC survivors who received both modalities. While there seems to be mixed findings regarding the duration of cognitive impairment associated with chemotherapy, research has established that not only is the administered dose pertinent, but also relevant is the use of any adjuvant therapies as well as hormonal replacement therapy.

Other Mediating Factors

Fatigue, cognitive dysfunction, and symptoms associated with an accelerated menopause are all possible chronic adverse effects of adjuvant chemotherapy for BC survivors. The interrelationships between them, as well as their impact on quality of life (QOL) have been investigated. In particular, Tchen and colleagues (2003) explored the incidence and severity of cognitive dysfunction, fatigue, and menopausal symptoms in women receiving adjuvant chemotherapy for BC compared to healthy controls matched on age, education, and marital status. Their findings revealed a higher incidence of moderate-to-severe cognitive impairment in the BC chemotherapy group compared to control participants. However, the authors' primary

hypothesis that cognitive dysfunction results from the fatigue and menopausal symptoms associated with chemotherapy was not supported. While they noted that fatigue has been associated with cognitive dysfunction in other populations such as chronic fatigue syndrome (Smith & Sullivan, 2003), and that fatigue is commonly reported by most BC patients, both during and after adjuvant chemotherapy, in this neuropsychological study, only fatigue, menopausal symptoms and QOL were strongly correlated. None of these factors correlated with cognitive dysfunction. Moreover, these same authors completed a one and two year follow-up study reporting that while fatigue, menopausal symptoms and cognitive dysfunction were indeed important adverse effects of chemotherapy for the BC group, they also tended to diminish over a two year period for most BC patients.

The aforementioned Brezden study (2000) also investigated if mood disturbances might have contributed to any cognitive differences found between groups considering the possible stress associated with diagnosis and treatment. No significant mood differences were observed between the two groups suggesting variables such as anxiety and depression did not play a role in their findings. At the same time, it is recognized that many neuropsychological tests lack ecological validity. For example, Shilling & Jenkins (2006) found little correlation between self-report measures and scores on neuropsychological tests investigating chemo fog in BC patients. Thus, trying to assess real-world impact, Calvio and colleagues (2010) investigated performance-based and patient-reported cognitive limitations on work output for active BC survivors. On average, three years post-treatment, they found self-reported distress, fatigue, job anxiety, and cognitive difficulties to be higher in the BC survivor group compared to a non-cancer control group. These findings suggest that other factors such as fatigue and stress may be partially responsible for any cognitive decline experienced by BC patients. In fact, some studies have

documented a high positive correlation between stress and cognitive disturbances (Castellon, S.A. et al., 2004; Schagen, S.B. et al., 2002). Equally important, stress and fatigue may confound the problem pre-treatment. Cognitive testing completed just prior to chemotherapy may not provide a true index of premorbid abilities due to the emotionally demanding experience of the diagnosis alone.

Indication for Additional Research

As recently as 2010, Vardy and Dhillon declared that much of the extant literature does not link self-reported memory symptoms and neuropsychological test scores. This discrepancy is unfortunate as it can lead to BC survivors feeling misunderstood, and may lead to a lack of self-reported difficulty if medical professionals and insurance companies dismiss their concerns. Given the variability in results of neuropsychological investigations of chemo fog, it is clear that there is a need for additional research to better understand this phenomenon. Perhaps inconsistent methodologies amongst researchers play a role in the conflicting findings, or it is possible that standard neuropsychological measures are not sensitive enough to detect the more subtle cognitive changes. Ouimet et al. (2009) completed an analysis of statistical models used in measuring neuropsychological change following BC treatment. Findings stressed several important methodological issues for consideration: clear definition of cognitive impairment to facilitate comparison and replication across studies, selection of an appropriate control group to minimize confounding variables, a psychometrically sound test battery that focuses on function previously identified in the literature as impaired in order to minimize statistical error, and prospective designs that assess premorbid function at baseline for measuring decline.

Even slight cognitive changes can be of great concern to those individuals with high premorbid abilities (Nieuwenhuijsen, K., et al, 2009; Clegg, E., 2009; Silverman, D & Davidson,

I., 2009). Attention to rigorous research methods is critical given the discrepancy between BC research into chemo fog and the experiences reported by BC patients. In a vocation that demands high cognitive functioning, these individuals may face challenges upon their return to work. In summary, the field of neuropsychology has contributed significantly to our understanding of chemo fog over the past 10 years. It has demonstrated the importance of critical factors such as the dose of chemotherapy, the number of cycles, adjuvant therapies, impact of menopause, and well-being variables (mood, anxiety, fatigue). Without dismissing the richness and complexity of the aforementioned literature, for the purposes of this thesis, in the next section we explore the neuroimaging BC literature.

Neuroimaging Research

Electroencephalogram (EEG)

In addition to the neuropsychological research, a handful of imaging studies have also investigated chemo fog in the BC population in hopes of better understanding the phenomenon. Kreukels and colleagues (2006) combined EEG and neuropsychological measures to examine more closely the mechanisms underlying cognitive deficits in BC patients treated with adjuvant chemotherapy. Specifically, they investigated reaction times as well as the amplitudes and latencies of the P3 wave, a known electrophysiological index of information processing. While performing an information-processing task and concurrently monitoring brain activity with EEG, 29 BC patients treated with chemotherapy (17 with conventional-dose chemotherapy, 12 with high-dose) were compared to 23 control participants with Stage I BC not treated with chemotherapy. The average time since treatment was 3.7 years. Findings indicated a significant overall reduction in amplitude of the P3 component in the high-dose chemotherapy group compared to the non-chemotherapy BC group only. No group differences were found in reaction

times. Unfortunately, the study ran into a number of difficulties, including a high error rate in one part of the task that resulted in the removal of that condition from the model. Additionally, other factors may have had an effect on observed findings, for example the level of education for the high dose group being much higher than that of the other two groups, and substantial variances in menopausal status and tamoxifen use amongst the three groups. However, building on findings in their earlier study (Kreukels et al., 2005) that showed electrophysiological differences between BC patients treated with standard chemotherapy and BC patients who had local treatment only, these investigators focused on the attention processing in these patients. Indeed, the P3 component has been validated in assessing high-level attention processing (Donchin & Coles, 1988). Several hypotheses were offered regarding their current findings. Included was the fact that the P3 reduction observed in the high-dose chemotherapy group might indicate a loss in information transmission due to their inability to properly focus their attention toward important stimuli. This interpretation extended from the idea that variations in amplitude are thought to be related to the intensity of information processing and whether or not proper allocation of such resources has been made. Nonetheless, due to the aforementioned difficulties, any claims of suboptimal neural functioning would require further investigation.

Positron Emission Tomography (PET)

Another group of researchers used PET combined with neuropsychological testing to compare 16 BC patients treated with adjuvant chemotherapy (five to ten years previously) to eight control subjects who had never received chemotherapy, but to whom five of which had a history of BC (Silverman et al., 2007). Altered metabolic activity was found in the inferior frontal cortex during performance of a short-term memory word recall task in the chemotherapy treated BC participants. During the same task, performance for the group of untreated BC

participants showed a large amount of activity in both the parietal and occipital cortices. At the same time, women receiving tamoxifen in addition to chemotherapy demonstrated low basal ganglia activity compared to those whose treatment regime was restricted to chemotherapy, and to controls who had no exposure to either treatment. While this study offers evidence for neural changes in those BC patients who have undergone chemotherapy, it also brings into question the effects of more than one intervention.

Magnetic Resonance Imaging (MRI)

Over a decade ago, Brown and colleagues (1998) were studying white matter changes induced by high-dose chemotherapy using MRI in a very small sample of eight BC participants. All eight participants were randomly selected from a pool of patients diagnosed with Stage II to Stage IV BC, who were concurrently involved in a bone marrow treatment program, thus receiving autologous hematopoietic progenitor cell support (AHPCS). Importantly, not only was the design longitudinal (involving repeated measures), but the first MRI for all eight participants took place prior to induction chemotherapy (the very first cycles of chemotherapy). However, only six of the eight participants had MRIs during induction chemotherapy, and only four had MRIs at months 10 to 13, after exposure to high-dose chemotherapy. Nonetheless, before induction chemotherapy, all eight participants showed no white matter abnormalities, other than nonspecific findings typical for age. These findings remained stable for all six participants (who continued in the study) after completion of induction chemotherapy, but before exposure to high-dose chemotherapy. At three months after high-dose chemotherapy, three of the four remaining participants showed increasing white matter changes, which then stabilized (no white matter abnormality) at six months post high-dose chemotherapy and up to one year after completion of high-dose chemotherapy. The authors concluded that MRI results demonstrated that white matter

changes do occur shortly after the conclusion of high-dose chemotherapy in BC patients receiving AHPCS, but also seem to stabilize within one year post high-dose chemotherapy. While these findings are intriguing, one must be careful in interpreting such results due to small sample size and the inclusion of the AHPCS protocol.

More recently, Yoshikawa et al. (2005) used MRI to examine the effects of adjuvant chemotherapy on hippocampal volume of BC survivors who were at least three years post surgery. The hippocampus, located in the medial temporal lobe, is a principle cortical structure within the limbic system that not only plays a crucial role in memory, but is vulnerable to the effects of stress. There were no significant differences in hippocampal volume between the two groups (44 BC participants who had received adjuvant chemotherapy and 31 BC participants who had not). Interestingly, during the neuropsychological testing component of the study, there were significantly lower attention/concentration scores for the adjuvant chemotherapy group, although the effect size was small. Due to study design (lack of premorbid measure, inclusion of numerous chemotherapy regimens), as well as reported rates of tamoxifen therapy and post-menopausal states being significantly higher among those who underwent chemotherapy, the effects are difficult to interpret; therefore generalization of results beyond the sample group is not possible. As these authors noted, future neuroimaging studies might help to elucidate the pathophysiology of memory impairment in BC survivors.

In a follow-up study, several of these same researchers went on to use MRI to explore possible differences in regional brain volume of BC survivors exposed to adjuvant chemotherapy compared to those who had not (Inagaki et al., 2007). Data from two BC survivor databases of brain MRI scans showed smaller gray and white matter within the prefrontal, parahippocampal, cingulate, and precuneus areas for the BC chemotherapy group compared to the non

chemotherapy BC group. Furthermore, the volumes of the prefrontal cortex, parahippocampal gyrus, and precuneus correlated significantly with performance on tests of attention/concentration and/or visual memory. However, deficits were not present three years post chemotherapy intervention. Again, this study faced similar biases as previously mentioned for this same group of investigators. Nonetheless, their findings do suggest that brain volume changes exist in BC survivors who have been exposed to adjuvant chemotherapy, but that they are only temporary in nature.

Similar findings were reported by McDonald et al. (2010) whose research did include a pre morbid measure and compared women undergoing treatment for BC, with and without chemotherapy, and a healthy control group. These researchers found no between-group gray matter differences at baseline, decreased gray matter density in bilateral frontal, temporal and cerebellar regions as well as the right thalamus one month post chemotherapy, with recovery observed at the one year post chemotherapy mark in some regions but not all. Recovery was observed in the following areas: bilateral superior frontal, left middle frontal, right superior temporal and in the cerebellar regions. Findings were reported in regions associated with cognitive functioning; thus the fact that some areas did not fully recover at the one year mark may be indicative of cognitive impairment at this time. Additionally, findings linking structural brain changes post chemotherapy and declined cognitive functioning were replicated in a follow-up study by this same group using data from a larger, more demographically diverse sample (McDonald et al., 2012). These later observations represent an interim analysis for which the final study visit data have yet to be reported, not allowing for a conclusion of duration of impact. Our own group recently published findings showing pre chemotherapy neuroanatomical differences between BC patients and well matched controls using voxel-based morphometry

(Scherling et al., 2012). Again, further analyses are required to report on change over time in this sample.

Using a different structural MRI technique called diffusion tensor imaging (DTI), Deprez et al. (2011) investigated chemotherapy-induced white matter changes in BC patients. These authors explored the correlation between impaired cognitive functioning in BC patients and chemotherapy-induced structural changes in cerebral white matter. DTI allows researchers to examine the integrity of white matter fiber tracks. Damage to axonal pathways will result in changes in quantitative DTI parameters, including fractional anisotropy (FA) and mean diffusivity (MD). The former describes the directionality of diffusion while the latter describes the average amount of diffusion (Pierpaoli and Basser, 1996). Results indicated significantly decreased FA in frontal, fronto-occipital and temporal white matter tracts for the group of chemotherapy treated BC patients compared to both healthy controls and a group of non-chemotherapy treated BC patients. The inclusion of the latter group was an effort to control for the diagnosis of cancer itself and the role it might have on cognitive functioning, however it should be noted that all but one from this group received radiation therapy and many were administered hormonal therapy. The total sample included 17 post chemotherapy intervention BC patients (between 80-160 days after completion), 18 age-matched healthy controls and 10 non-chemotherapy treated BC patients. Self-reported subjective cognitive complaints of BC patients also correlated negatively with FA in frontal and parietal regions. Lastly, it was reported that the observed decrease of FA in both temporal and parietal white matter tracts was significantly associated with lower performance on neuropsychological tests of attention and processing speed. However, while age, major depression, and anxiety were all controlled for, education level was not. Although all participants had received education until a minimum of 18

years of age, participants were not matched on this variable, thus presenting a potential confound in terms of findings for the cognitive assessment.

Another group of researchers (de Ruiter et al., 2012), using this same technique, also found DTI values indicative of reduced white matter integrity nine years post chemotherapy compared to a group of BC survivors who had not received chemotherapy as part of their treatment. Yet as seen in many of the studies previously discussed, data for both DTI investigations were acquired post chemotherapy, thus any premorbid deficits would not have been studied and it is impossible to interpret findings as attributable to chemotherapy alone. However, Deprez and others (2012) addressed this shortfall and compared DTI data obtained before and after chemotherapy, showing significantly decreased FA after chemotherapy in frontal, parietal and occipital areas for the BC chemotherapy treated patients only. Clearly the aforementioned studies offer some evidence for a reduction in white matter tracts of those BC patients exposed to chemotherapy compared to non-chemotherapy treated BC individuals as well as healthy controls, while substantiating a significant correlation between white matter reduction and impaired cognitive functioning in BC patients. At the same time, interpretation of BC imaging research to date stresses the need for a fuller understanding of the cognitive impairment associated with chemotherapy.

Functional Magnetic Resonance Imaging (fMRI)

Cognitive deficits are common after chemotherapy, but have not been documented systematically. Study design, including varied control groups, task administered, analyses performed, and measures collected have contributed to this lack of consistency in the literature. The poor correlation between self-report measures and scores on neuropsychological tests that investigate chemo fog in BC patients is also noteworthy (Shilling & Jenkins (2006). Most

researchers agree that the etiology behind the decline in cognitive functioning of BC survivors treated with chemotherapy is poorly understood. This has led to the lack of credibility in BC patient complaints of chemo fog, and supports the need for further research into its neural correlates. Empirical evidence is required to substantiate chemo fog as a brain-based phenomenon if it is to be acknowledged as a legitimate concern by physicians and therapists alike. This is an area of investigation for which fMRI is well suited. fMRI is a non-invasive neuroimaging technique that measures blood flow in the brain while a person is performing a task (behavioural, cognitive or emotional). It is a safe and sensitive tool for detecting changes in brain function that may otherwise go undetected in traditional neuropsychological tests (see Measures section, pg. 28 for detailed fMRI description).

To date, little fMRI research has been performed to investigate the cognitive deficits associated with chemotherapy in BC survivors. In 2007, Ferguson and colleagues presented a case of monozygotic twins discordant for BC. One twin had no history of cancer, whereas the other twin underwent chemotherapy for Stage II BC 22 months before enrolling in the study. Both twins were evaluated with standardized self-report measures of cognitive function and neuropsychological tests, as well as structural (MRI) and functional (fMRI) imaging. Although only small differences were detected by standard neuropsychological measures, significant contrasts were found in the self-reported cognitive complaints and in both structural and functional MRI images. Findings revealed the twin who underwent chemotherapy had substantially more subjective cognitive complaints, more white matter hyper-intensities (MRI), and greater brain activation (fMRI) during working memory processing compared to the non-affected twin. While performing an fMRI N Back working memory task, the twin with BC demonstrated a much broader scope of activation in known typical working memory circuitry

(i.e., bilateral frontal and parietal regions). With no reported difference in task performance accuracy, it is possible that the BC twin was recruiting additional brain areas in a compensatory effort likely due to an impairment in working memory functioning.

Subsequently, de Ruiter and colleagues (2010) used fMRI along with neuropsychological testing measures to examine long-term cognitive impairment in BC survivors. Executive function and episodic memory performance was measured in both ten year post high-dose adjuvant chemotherapy BC survivors and BC survivors who had no chemotherapy intervention. For those who received chemotherapy, fMRI revealed hypo-responsiveness of the dorsolateral prefrontal cortex in the executive functioning task (Tower of London) and of the parahippocampal gyrus during assessment of episodic memory (Paired Associates task). These hypo-activations were accompanied by a significantly diminished performance of the chemotherapy group compared to the control group. Though accuracy over speed was emphasized, on average members of the chemotherapy group responded more quickly than did members of the control group, suggesting difficulties in response inhibition for this group. Moreover, they found neuropsychological testing showed a relatively stable pattern of cognitive impairment in the chemotherapy group over time by comparing current performance to previously acquired scores (which varied from less than two years to more than nine years after chemotherapy.)

Although these studies are interesting and suggest a role for fMRI in the study of chemo fog, they also confirm the need for more elaborate study designs that include pre treatment imaging and control of multiple lifestyle variables. Indeed, Cimprich et al. (2010) have shown that even before chemotherapy there are differences in cognitive performance between women with BC and those without cancer. Using fMRI, these authors reported that both groups showed bilateral brain activation in high-demand task conditions, but the BC group also recruited

additional components of attention/working memory circuitry in both hemispheres compared to controls. Findings demonstrated significantly decreased accuracy and slower task performance for the women with BC prior to chemotherapy compared to the performance of the control group. Specifically, the BC group showed significantly less accuracy in the high-demand condition of the verbal working memory task than did the cancer-free controls. Our group (Scherling et al., 2011) has also published findings that demonstrated pre chemotherapy differences between BC patients and matched controls during performance of a visuospatial working memory task. Even more recently, McDonald et al. (2012) found significant group differences pre chemotherapy for BC patients compared to healthy controls using a high load working memory task (3-back). These studies of compromised cognitive neural functioning in BC patients before any chemotherapy treatment indicate the need for further research.

Another important aspect of chemo fog study design includes the contribution of stress and other lifestyle variables on brain functioning. Findings may be related to the diagnosis itself, and it is also possible that fatigue, mood and high stress contribute to the imaging results from BC patients. Results published by Scherling et al., (2011) and Lopez Zunini et al., (2012) certainly highlight the importance of controlling for such factors as depression, anxiety and fatigue.

Current Study

The purpose of the work described in this thesis is to further our understanding of the neural underpinnings of chemo fog in the BC population. Using a quasi-experimental design, and building on previous research, this study used fMRI to address some of the short-comings in the BC literature. For example, this is one of the first fMRI studies to measure neural processing

both before and after chemotherapy in BC patients compared to a well matched control group while controlling for many potentially confounding variables. More specifically, we investigated one of the most prevalent complaints associated with chemo fog in the BC population: diminished working memory.

The construct of working memory is defined as the processes involved in temporarily storing, manipulating, and retrieving information during the performance of complex cognitive activities. One commonly discussed theoretical model of working memory is from Baddeley (1986). According to Baddeley's theory of working memory, three major components are involved: the phonological loop, the visuospatial sketchpad, and the central executive. The first two, phonological loop and visuospatial sketchpad, are passive slave systems under the control of the central executive. The phonological loop is a short-term memory store requiring the temporal lobe (Wernicke's area), which holds traces of acoustic, or speech based material. The visuospatial sketchpad is responsible for the manipulation and temporary storage of visual and spatial information processed in what is known as the ventral (object recognition) and dorsal (how/where) pathways of the brain (extending from the occipital lobe to the temporal lobe in the former, and extending to the parietal lobe in the latter). The most important component of Baddeley's working memory model is the central executive, focussed in the dorsal lateral prefrontal cortex (DLPFC). The central executive determines what to do with the contents of the phonological loop and visuospatial sketchpad, i.e., maintain for additional processing, direct into long-term memory storage, or discard. Ultimately, its role is to keep the two slave systems organized, but also allows for relevant information to be retrieved from long term memory into the working memory system for online processing. A meta-analysis conducted by Smith et al. (1998) revealed that the N Back task, commonly used in neuroimaging studies is valid for

assessing aspects of working memory. There are several variations to the N Back task, but for this study a Letter N Back and a Visuospatial N Back task were used (see Methods Pg. 29 & Appendix A for complete task description/sample).

Furthermore, this study design contributes to a better understanding of chemo fog in BC patients through the use of a well matched control group, by using a longitudinal design and by controlling for several potentially influential variables, such as anxiety, mood, and fatigue. This study combined the use of fMRI, and several measures of state of being from self-report questionnaires, before, right after and one year following chemotherapy. It was hypothesized that fMRI would reveal significant differences in patterns of blood flow between controls and patients before, right after and one year after chemotherapy as they performed working memory tasks. It was also hypothesized that the BC patients' neural activity would alter depending on their time since treatment. It was expected that the largest differences would be observed right after chemotherapy and be anatomically related to Baddeley's working memory model. It was also posited that other factors (mood, anxiety, fatigue) would contribute to neural processing in the women with BC.

Method

Participants

This study compared a group of women with early stage BC attending the Ottawa Hospital Regional Cancer Centre (TOHRCC) to a group of healthy women matched on age and education ($n = 19$ in both groups). Originally there were twenty-three participants per group, but four withdrew from the study: One BC patient had a re-occurrence and therefore was no longer eligible, while the other three withdrew due to personal reasons. fMRI power calculations suggest that for a threshold of $p = 0.05$, approximately 12 subjects are required to achieve 80%

power at the single voxel level (Desmond, 2002). Thus, sample size was adequate to detect differences (i.e., should the null hypothesis be false). This thesis focused on the fMRI component of a larger study that involved psychometric testing, fMRI during two other tasks, and structural MRI. For additional details on psychometric testing, see Appendix B. Results of these tests will be addressed in the discussion as they pertain to the fMRI results.

The patient group comprised women who had initially undergone a mastectomy or lumpectomy for Stage I or II BC, followed by chemotherapy intervention. Various anthracycline-based chemotherapy regimens were acceptable for inclusion. It was projected that approximately half of the patients in the sample would receive adjuvant hormonal therapy based on statistical data collected in TOHRCC data base. Patients were not eligible if they commenced hormonal therapy prior to completing chemotherapy.

It was estimated that eight to ten new BC patients would be seen in TOHRCC each week according to discussions held with the senior oncologist at this site. Researchers were given contact information of interested participants only in order to explain the study protocol as well as gain informed consent if the participant was willing. (See Appendix C for Informed consent/study rationale). Women were asked to nominate a healthy female peer of approximately the same age and education level to serve as a control participant. If she was unable to do so, one was recruited for her (through BC clinic website advertisement). Matched controls participated in the same testing procedures as the index patient.

All participants were between the ages of 18 and 65 years (to reduce the risk of age-associated cognitive decline), fluent in English (tests used were not available in French) and had at least a grade eight education. Any history of previous cancer, chemotherapy, radiotherapy, serious psychiatric or neurological illness, or significant substance abuse was grounds for

exclusion from the study. To assist in screening out serious psychopathology, a Beck Depression Inventory II (BDI-II) score of > 20 (Beck et al, 1996) and/or a Beck Anxiety Inventory (BAI) score of > 15 (Beck & Steer, 1990) were also used as exclusion criteria. Additional fMRI specific exclusion criteria included left-handedness, claustrophobia, and having a pacemaker or ferromagnetic metal implants. Data from any participant were excluded from analysis if, over the course of the study, she developed inter-current cancer or any illness that could affect cognition.

Based on previous research with this population, it appears that recruitment of participants is most efficient if study details are provided by a patient's oncologist and then followed by a clinical trials coordinator (or oncology nurse) from the TOHRCC. Thus, our own coordinator identified all new referrals of BC patients and endeavoured to attend all medical oncology clinics where new BC patients were seen. Recruitment was pre-surgery, baseline fMRI was administered on average one month after surgery, while the second scanning session was administered approximately one month following the last chemotherapy cycle of the BC patient. The third and final fMRI session took place one later. Optimization of participant retention was obtained by 1) keeping the testing battery as short as possible; 2) making every effort to accommodate the schedules of participants (e.g., seeing them at the time of their choice; providing parking); 3) providing a \$30 stipend for each study session; 4) providing each patient with a copy of her MRI structural scan, as well as with the results of the study, once available; and 5) making every effort to ensure that participants' study experience was a validating and enriching one. Approval for this study was obtained from both the Ottawa Hospital Research Ethics Board and the University of Ottawa Research Ethics Board. Funding was secured from the Canadian Breast Cancer Foundation, Ontario Chapter to fund this study in its entirety (both

neuropsychological and fMRI measures). First imaging scans were administered in the fall of 2008 and the last one was completed in February of 2009.

Measures

MRI and fMRI

To understand fMRI data, MRI mechanics must be understood. MRI defines the anatomical structure of the human brain, and is based on the phenomenon that the longitudinal axis of hydrogen nuclei (prevalent in the human body due to the high water content) will change from random to parallel orientation when placed in a magnetic field. To produce an MR image, radio wave pulses are then applied to the brain forming a second magnetic field. This radio wave energy pulse momentarily rotates the nuclei away from the parallel orientation. If the pulse is turned off, the nuclei return to parallel alignment within the magnetic field. When the different types of tissue come back into parallel alignment, known as relaxation, energy transferred to the nuclei is then released. The rate of energy release is recorded as current. Each type of tissue (i.e., white matter, gray matter, cerebral spinal fluid, tumours or infarctions) releases a distinct current level when it returns to the relaxed or lower energy state. These distinct current levels produce high spatial resolution images of the anatomical features of the brain (Huettel, Song & McCarthy, 2009).

fMRI uses the same scanner that produces structural images, but fMRI differs in that it measures blood flow in the brain while a participant is performing a task (behavioural, cognitive or emotional). It is a non-invasive technique as no injections are required. fMRI relies on the naturally occurring Blood Oxygen Level Dependent (BOLD) effect. The BOLD effect is an indirect measure of neuronal activity. When neurons are at rest, there is more deoxyhemoglobin than oxyhemoglobin present in the surrounding brain region. As neuronal activity increases (e.g.,

the brain is activated) the level of local oxygenated blood, oxyhemoglobin, increases, replacing the deoxyhemoglobin. The oxyhemoglobin increase produces an increase in magnetic signal which is then quantified with fMRI measurements. Whether an area is active or not depends on the magnitude of change in this oxyhemoglobin. fMRI can be repeated on the same individual without concern and enables researchers to examine the human brain in action (Ogawa et al., 1990).

In terms of construct validity, fMRI measures changes in blood flow within the brain, thus its relation to neuronal activity is correlational. It is thought that changes in brain blood oxygenation levels are a result of task demands. Based on this assumption, researchers can localize brain activity on a second by second basis within millimetres of its origin. As such, fMRI studies assemble maps that link brain activation (e.g., changes in blood flow) to hypothesized mental function. The reliability of fMRI data involves detecting a meaningful signal (what the researcher is attempting to measure) while decreasing the noise (confounds such as subject heart rate and movement). Good fMRI design maximizes signal intensity to increase the quality of data (e.g., through choice of task and data processing). Many fMRI reliability studies have estimated the test-retest reliability of fMRI data to be quite high with an average intra-class correlation coefficient value reported at 0.50 (Duncan et al., 2009).

fMRI N Back task

The Letter N Back and the Visuospatial N Back tasks involve multiple processes attributed to the working memory system: encoding, monitoring, maintenance, updating, and matching of the current stimulus to the one that occurred N presentations back. These tasks have been designed with one, two, three, and four back conditions. This thesis used the two back condition as a measure of working memory processing for both the Letter and Visuospatial N

Back tasks (LNB; VSNB). Several studies have demonstrated reliability for these tasks, consistently showing activation changes mainly in bilateral prefrontal and parietal cortices, with test-retest reliability estimates exceeding an r of .80 (Jaeggi et al., 2010).

The stimuli for the LNB task were letters presented one at a time in the center of the screen and the conditions were 'Press for X', pressing the button every time an 'X' was presented (baseline) and 'Press for 2-back', pressing when the letter was the same as the letter presented two trials prior (test) (2-back task). Stimuli were presented for one second and each block was 30 seconds. Four blocks of each condition were presented in a counterbalanced pattern and 21 second rest blocks were interspersed between test and baseline blocks where no response was required and no stimuli were presented. The duration of this task was seven minutes.

The paradigm for the VSNB task consisted of the letter 'O' presented in white on a black background, one at a time in nine different positions on a screen located at the entrance of the magnet bore. There were rest periods of 18 seconds each at the beginning, middle, and end of the task. The word 'Rest' was presented on the screen during these rest periods and no responding was required. The baseline condition began with the instructions 'Press for Centre O' on the screen for 3 seconds. Each time the 'O' was presented in the middle of the screen a button response with the right index finger was required. The instructions 'Press for 2 Back' were presented for three seconds at the start of each test condition block and subjects were required to press every time the 'O' was presented in the same position as it was in 2 presentations before. Baseline and test conditions were comprised of twenty-one stimuli, presented for one second every 2 seconds. The duration of this task was nine minutes. (See Appendix A for task samples).

Participants also performed two other tasks as part of the larger study (a response inhibition task as well as a word list task). However, these tasks are being analyzed as part of another thesis.

Procedures

Each fMRI session took place at a private MRI clinic in Hull, Quebec. All sessions lasted approximately 45 minutes and captured high-resolution structural images, as well as functional images while the subject performed cognitive tasks in the scanner. Patients and matched controls were scheduled for baseline (pre chemotherapy) fMRI scanning sessions (T1) once informed consent was obtained. Participants underwent a second fMRI session, identical to the first, within two to three weeks following the last chemotherapy cycle (T2). Participants were reassessed using MRI/fMRI one year after the completion of chemotherapy (T3). Participants in the control group followed the same time line as the patient to whom they had been matched. At the one year post chemotherapy scan, it was estimated that approximately half of the BC patients would be receiving hormonal therapy for up to one year based on figures recorded in TOHRCC database. However, records showed that hormonal therapy was commenced by almost all fMRI participants (only three did not), so we were not able to compare participants who received hormonal therapy to those who did not.

Anxiety and mood scores from the self-reported Profile of Mood States (POMS; Lezak, M., et al., 2004) were used to explore the role of stress and mood on working memory at each time point. The Anxiety subscale and Total Mood score were used accordingly. Lezak (2004) reports the POMS reliability and validity as adequate (test-retest $r = .39$). Due to our interest in investigating the impact of fatigue on brain activity and chemo fog, we assessed this measure for all three time points in BC patients using scores from the Functional Assessment of Cancer

Therapy- Fatigue scale (FACT-F; Cella, D., 1997). Cella, D., (1997) reports test-retest reliability for the FACT-F as good with an $r = .87$ and that both convergent and discriminant validity testing revealed a significant positive relationship with other known measures of fatigue.

fMRI Scan Procedure

Whole brain echo planar fMRI was acquired on a 1.5 Tesla Siemens Magnetom Symphony MR scanner with a pulse sequence of TR/TE 3000/40 ms, flip angle 90° , FOV $24 \times 24 \text{ cm}^2$, 64×64 matrix, slice thickness five mm, 27 axial slices, and bandwidth 62.5 kHz. At the MRI site, fMRI compatibility was ensured for each participant. In a separate room from the scanner, participants were given a demonstration of the tasks to be performed to ensure that they understood the instructions, were aware of what they would experience, and were able to perform the tasks. Following this training session, participants were placed in the scanner as per the standard head MRI protocol. The participant laid flat on an automated bed which was then introduced into the scanner, a two-foot wide cylinder. A blanket was offered to increase participant comfort levels. An MRI compatible fibre optic response device (Lumina LP-400 four button pad from Cedrus Corporation) was placed in the right hand of the participant to record task performance. The investigator placed the participant's right index finger on the appropriate response button indicating which key to use for the fMRI tasks. Stimuli for all fMRI tasks were presented by an LCD projector and computer in the control room onto a back projection screen located at the end of the patient table. All stimuli were white on a black background. A mirror on the head-coil allowed participants to view the stimuli. All lights were off throughout the imaging to enhance the contrast of the stimuli. Auditory communication between tasks was permitted by MRI compatible headphones and through the MRI audio system. No auditory stimuli were

presented. The first 15 minutes of each scanning session was dedicated to structural scans. A picture of water lilies was presented on the screen at this time to help relax the participant.

Following the collection of structural scans, the participant performed two tests of working memory while in the scanner, the Letter N Back task and the Visuospatial N Back task (described above with visual depiction in Appendix A). Presentation of the tasks were in a block design through the use of E-Prime. Transitions between conditions (blocks) represented changes in the level of the independent variable (working memory requirement). Between blocks, a rest period was presented so that brain activation independent of the two conditions was measured and the hemodynamic response returned to baseline between conditions. The time needed to complete both tasks was approximately 14 minutes. Time in the actual scanner was approximately 45 minutes.

Data Analysis

The Statistical Package for the Social Sciences (SPSS, version 19.0) was used to analyze all performance data (reaction times and errors) and well-being variables. SPM8 was used to pre-process (realign, normalize, and smooth) the fMRI data, and to perform the statistical analyses specific to these data.

Analysis of Performance Data and Well-being Variables

A three way mixed ANOVA design was conducted for all performance data for each task including that of errors of omission and commission and for reaction time. Group was the independent factor with two levels, and condition and time were both repeated factors with condition having two levels and time three levels. Additionally, a two by three mixed ANOVA design was conducted for both mood and anxiety, group being the independent factor with two levels, and time being the repeated one with three levels.

All data were screened for potential problems (outliers, distribution, impossible values, and skew) before statistical analysis. Performance parameters included reaction time as well as errors of omission and commission. Omission errors were defined as a failure to respond to a target stimulus and errors of commission included any response following a non-target stimulus. Mean reaction times were calculated for the Press for 2 back conditions of each task for all accurate responses occurring within 900 ms of stimulus presentation. Tasks were designed to optimize performance, thus emphasizing functional neural differences only. Due to this methodological approach, it was hypothesized that no group performance differences would be present. Using three independent group *t*-tests, 2 tailed, and an alpha level of 0.05, if significant differences were found between group means on any of the variables, they were used as covariates for the functional MRI analysis.

The same methodological approach was applied to both mood and anxiety well-being variables. However, due to patient cancer diagnosis, it was hypothesized that there would be significant differences found between group means on these two variables. Significant differences observed were also used as covariates for the functional MRI analysis. Fatigue was examined within BC Patients and will be addressed with the imaging data.

Analysis of Functional MRI

To decrease noise, maximize signal, and allow the magnetic signal to stabilize, the first nine seconds of images collected from each participant were discarded. Next, in order to prepare the data for statistical analysis, standard pre-processing steps were performed using SPM8 software to remove unwanted variability (noise) and to get the images into a suitable format for analysis. A major source of noise during fMRI data collection results from head movement in the scanner. Even with the use of a head coil during scan time, not all movement can be prevented.

Thus, images were realigned (motion corrected) using SPM8. This involved setting the first usable image as a reference image, to which all subsequent images were aligned. There were no images with head movements greater than three mm, thus all images were kept for data analysis.

Additionally, to account for significant variability in the human brain, images were normalized to compensate for any shape differences. Normalization compensates mathematically for differences in brain shape while attempting to minimize the differences between the subject's brain and the Montreal Neurological Institute (MNI) template provided by SPM8. Lastly, images were subjected to both temporal and spatial filtering, a pre-processing step referred to as smoothing. Images were smoothed to eliminate noise interference in the data and thereby increase the signal. This was achieved by smoothing over the transition points between two side by side activated voxels in the brain with a ten mm Gaussian kernel. Once completed, images were ready for first level analysis (fixed effects). The observed time course of image intensity in each voxel was temporally filtered to remove frequency components below 0.36 Hz and fitted (using the general linear model) to a model hemodynamic response consisting of sequential contributions from sequential epochs. The hemodynamic response to each type of epoch was modelled by a delayed, filtered boxcar waveform commencing six seconds after the start of the epoch and subjected to the same high pass temporal filter as the observed time course.

The goal of functional MRI is to relate changes in brain physiology over time to an experimental manipulation. fMRI data from each of the three imaging sessions were analyzed with SPM8 using: 1) fixed effects for individual participants, comparing the 2 back minus control conditions (referred to as the working memory contrast in the rest of this thesis) that produced the necessary contrast-image required for second level analyses; 2) random effects (known as second level analysis) for between group comparisons; including identified

covariates; 3) multiple regression to control for potentially significant effects of fatigue, mood and anxiety; and 4) both whole brain (statistical tests conducted on each voxel to evaluate significance) and region of interest (ROI) analyses (division of the brain into a smaller set of discrete regions which are then analyzed for significance). Each of these steps was used to compare both between group differences and within subject differences for: T1 vs. T2, T1 vs. T3 and T2 vs. T3 over the three imaging sessions. The T1 between group comparisons for the VSNB task is not reported as it has been published (Scherling et al., 2011).

The fixed-effects analyses included images for the working memory contrast for each task and for each participant. Each task was presented in blocks with two different conditions; control and test (Press for 2 Back). An average was calculated for the control condition which was then subtracted from an average calculated from the test condition resulting in a contrast image. Fixed-effects analyses presume that the experimental effect is constant across participants (fixed), other than the influence of random noise. Statistical inferences are thus restricted to the sample that the data were collected from. This model is subject to certain biases in that it relies on the mean value from all participants as its true effect, yet the manipulation may not affect all participants in the same manner. As such, summary statistics are calculated for each participant and used at the next level of analysis to make inferences about the population of interest. This next step, random effects analysis (also known as second level analysis in fMRI), includes information about the distribution of the effect across participants. Here, *t*-tests evaluate whether the participant's summary statistics are drawn from a distribution with a mean of zero. If found significant at the desired alpha level (set at 0.05 in this study), results are then generalizable to those similar to the sample of the study. The fixed effects analysis allows for inferences about the group of participants tested, whereas the random-effects analysis will allow for inferences to

be made about the population from which the participants are drawn. Both analyses are considered standard practices, and are necessary requirements.

Multiple regression was used to examine independent regressors. Both regressors of interest (fatigue, mood and anxiety), and head-motion parameters (known as nuisance regressors, or uninteresting sources of variability) were included in the design matrix. Using SPM8, at an alpha level of 0.05, it was determined how much each specified regressor predicted change in the BOLD signal. This examined the relationship between each regressor and neural activation patterns.

Whole brain and ROI analyses were conducted using an alpha level of 0.05. Multiple comparisons were corrected for by applying a Family Wise Error. Whole brain analyses are statistical tests on each activated voxel to evaluate its significance relative to the study's hypothesis. In contrast, ROI analyses partition the brain into distinct regions of interest to test for significance. ROIs are delineated by the Automated Anatomical Labelling (AAL) Atlas supplied in SPM. ROIs investigated in this study were determined a priori based on both functional and anatomical regions reported in the literature (working memory as well as in the BC population) specific to our hypotheses: bilateral frontal, temporal, parietal and cerebellar regions, including inferior frontal cortex, dorsolateral prefrontal cortex, medial temporal lobe, cingulate, and precuneus.

Results

Table one summarizes demographic and clinical characteristics for all participants. There were no significant differences between groups in age, education or pre-chemotherapy menopausal status. T-tests revealed that patients differed from controls in BDI scores, ($t(37) =$

2.54, $p < .02$) as well as BAI scores ($t(37) = 2.41, p < .02$) at baseline. The mean BDI score was higher in patients than in controls with Mean(Stdv) values being 9.47 (9.42) and 3.74 (3.16), respectively. Similarly, the mean BAI score was higher in patients 9.11 (7.58) than in controls 4.26 (4.44).

Performance Data Letter N Back Task (LNB)

Results of the LNB errors of omission analysis revealed that all main effects were significant and there was a significant three way interaction (Main effect of time, $F(2,64) = 19.414, p < .001$, main effect of condition, $F(1,32) = 51.842, p < .001$, main effect of group, $F(1,32) = 8.792, p = .006$, and three way interaction, $F(2,64) = 5.248, p = .008$). Pairwise comparisons revealed that patients at time three made significantly more errors of omission for the Press for 2 Back condition ($M = 5.529, SE = .745$) during working memory compared to controls ($M = 2.000, SE = .745$). Figure 1 shows the LNB Time x Condition x Group profile plot.

Results of the LNB errors of commission analysis revealed a significant main effect of condition ($F(1,32) = 25.753, p < .001$) and an interaction of time by group ($F(2, 64) = 3.415, p = .039$, but no main effect of time ($F(2, 64) = 1.144, p = .325$) or group ($F(1, 32) = .198, p = .659$). Pairwise comparisons revealed fewer errors in the Press for X condition ($M = .324, SE = .071$) compared with the Press for 2 Back condition ($M = .892, SE = .130$) as would be expected.

Results of the LNB reaction time analysis revealed a significant main effect of condition ($F(1, 32) = 103.440, p < .001$) and a significant three way interaction, ($F(2,64) = 3.760, p = .029$), but no main effect of time ($F(2,64) = .811, p = .449$) or group ($F(1,32) = 2.446, p = .128$).

Pairwise comparisons revealed faster reaction times for the Press for X condition ($M = 408.00$, $SE = 8.00$) than the Press for 2back condition ($M = 513.00$, $SE = 14.00$) as would be expected.

Performance Data Visuospatial N Back Task (VSNB)

Results of the VSNB errors of omission analysis revealed a significant main effect of both condition and group, and an interaction between time x group, condition x group and time x condition (Main effect of condition, $F(1,32) = 64.668$, $p < .001$, main effect of group, $F(1,32) = 4.400$, $p = .044$), and the following interactions; time x group, $F(2,64) = 5.383$, $p = .009$, condition x group $F(1, 32) = 6.935$, $p = .013$, time x condition -sphericity could not be assumed, therefore reporting Huynh-Fedlt, $F(2, 64) = 3.545$, $p = 0.36$. Pairwise comparisons revealed patients making significantly more errors of omission ($M = 5.353$, $SE = .838$) during working memory compared to controls ($M = 1.971$, $SE = .838$) at time 3. Figure 2 shows the VSNB errors of omission interaction between Time and Group.

No significant results were observed from the VSNB errors of commission analysis (Main effect of time ($F(2, 64) = .752$, $p = .476$), main effect of condition ($F(1, 32) = 1.702$, $p = .201$, main effect of group ($F(1, 32) = .446$, $p = .509$, and three way interaction $F(2, 64) = .707$, $p = .497$).

Results of the VSNB reaction time analysis revealed a significant main effect of condition and an interaction between time and condition (Main effect of condition, $F(1, 32) = 43.763$, $p < .001$; and interaction with corrections to sphericity, therefore reporting Huynh-Fedlt, $F(2,64) = 4.467$, $p = .015$). Main effect of time ($F(2, 64) = .326$, $p = .723$) and group were not observed ($F(1, 32) = 3.322$, $p = .078$). Pairwise comparisons revealed the control condition - Press for X ($M = 441.00$, $SE = 9.00$) giving rise to quicker response times compared to test condition - Press for 2back ($M = 517.00$, $SE = 14.00$) as would be expected.

Well-being Variables POMS-Mood

A significant main effect of group was revealed, $F(1, 36) = 9.844, p = .003$ with patients reporting significantly higher mood scores ($M = 31, SE = 7$) than controls ($M = .05, SE = 7$). Mood scores went from 32 to 38 to 24 over the three time points in the patients. However, no main effect of time ($F(2, 72) = 2.575, p = .083$) was revealed. Figure 3 shows main effect of group for mood, higher scores indicating greater mood disturbance.

Well-being Variables POMS-Anxiety

A significant main effect of group was revealed, $F(1, 36) = 7.095, p = .011$ with patients reporting significantly higher anxiety scores ($M = 10.5, SE = 1$) than controls ($M = 5, SE = 1$). Anxiety scores for patients went from 13 to 10 to 9 from T1 to T3. However, no main effect of time ($F(2, 72) = .108, p = .897$) was revealed. Figure 4 shows main effect of group for anxiety, with higher scores indicating a higher level of anxiety.

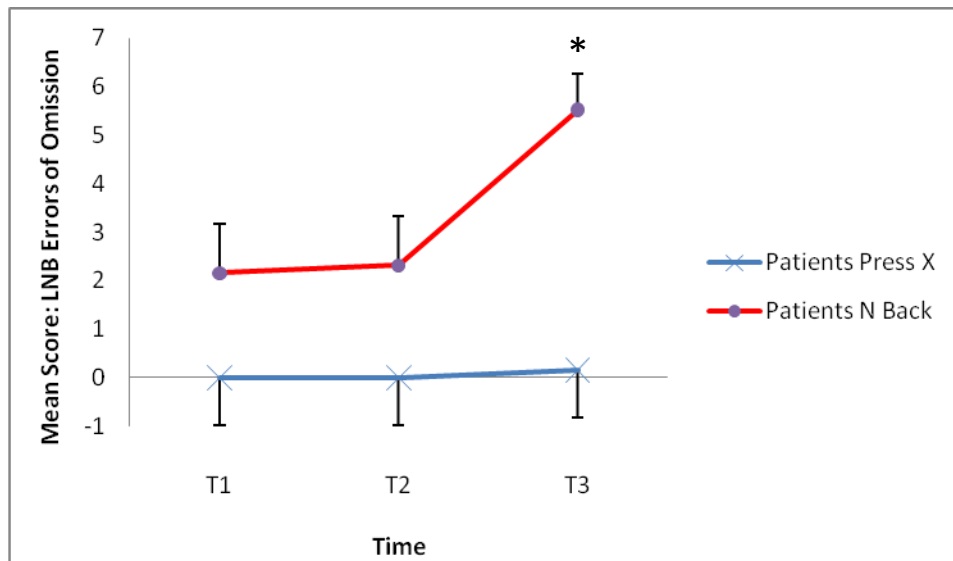
Table 1: Demographic and Treatment Characteristics of the Sample

Variable	Patients (<i>n</i> = 19)	Controls (<i>n</i> = 19)	p-value
Age	49.26 (7.58)	48.58 (8.48)	p>.83
Education Level			p>.61
High School	2	3	
College	9	4	
Bachelor	5	2	
Graduate degree	3	4	
Some post-secondary	0	6	
Marital Status			
Married/Common Law	17	10	
Single	0	3	
Separated/Divorced	1	6	
Widowed	1	0	
Cancer Stage			
1	3	-	
2	14	-	
3	2	-	
Menopausal Status Pre Chemotherapy			p>.68
Menstruating	7	9	
Perimenopausal	3	2	
Postmenopausal	9	8	
Beck Depression Inventory II- baseline	9.47 (9.42)	3.74 (3.16)	p<.001
Beck Anxiety Inventory-baseline	9.11 (7.58)	4.26 (4.44)	p<.001

Chemotherapy Regime	# of Chemotherapy Cycles	Patients per # of cycles
Fluororacil + epirubicin	7	1
+cyclophosphamide + docetaxel	6	10
	5	2
Fluororacil + epirubicin		
+cyclophosphamide + docetaxel	6	2
+ hepirubicin		
Cyclophosphamide + docetaxel	4	4

Figure 1: Letter N back Errors of Omission Three Way Interaction Plot

(Time*Condition*Group) - **Patients**



(Time*Condition*Group) - **Controls**

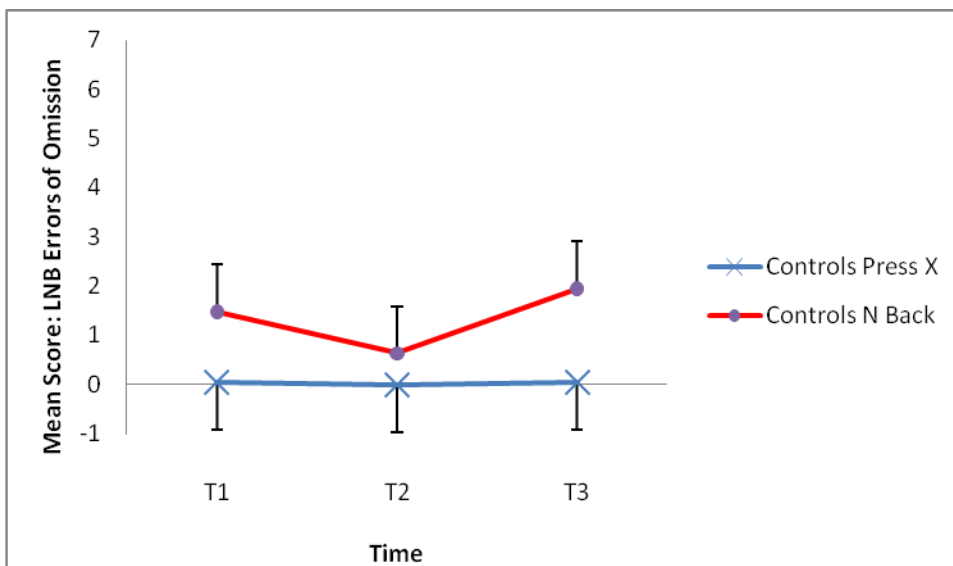


Figure 2: Visuospatial N back Errors of Omission Group x Time Interaction

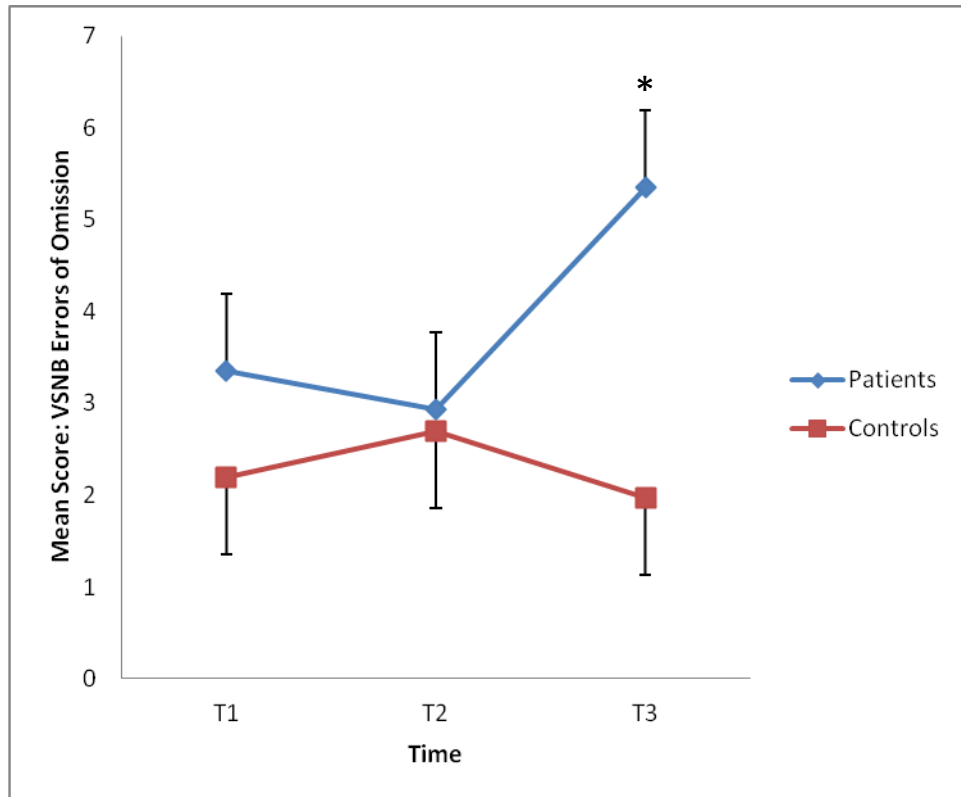


Figure 3: Wellness Variable Mood: Main Effect of Group Collapsed across Time 1, 2, & 3

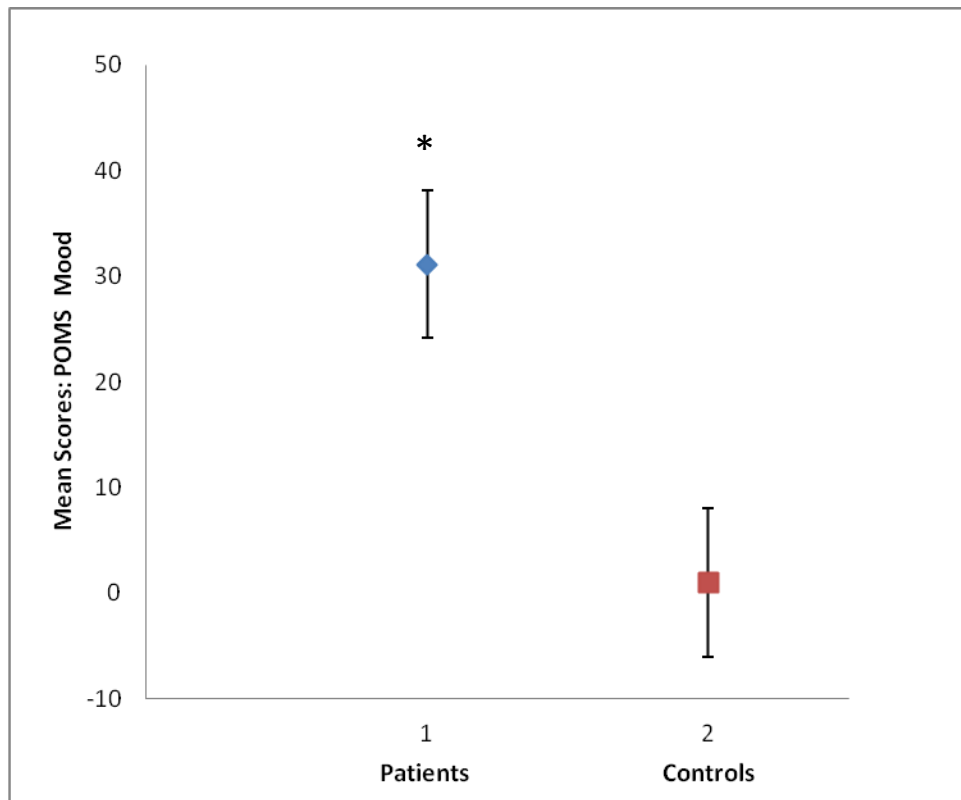
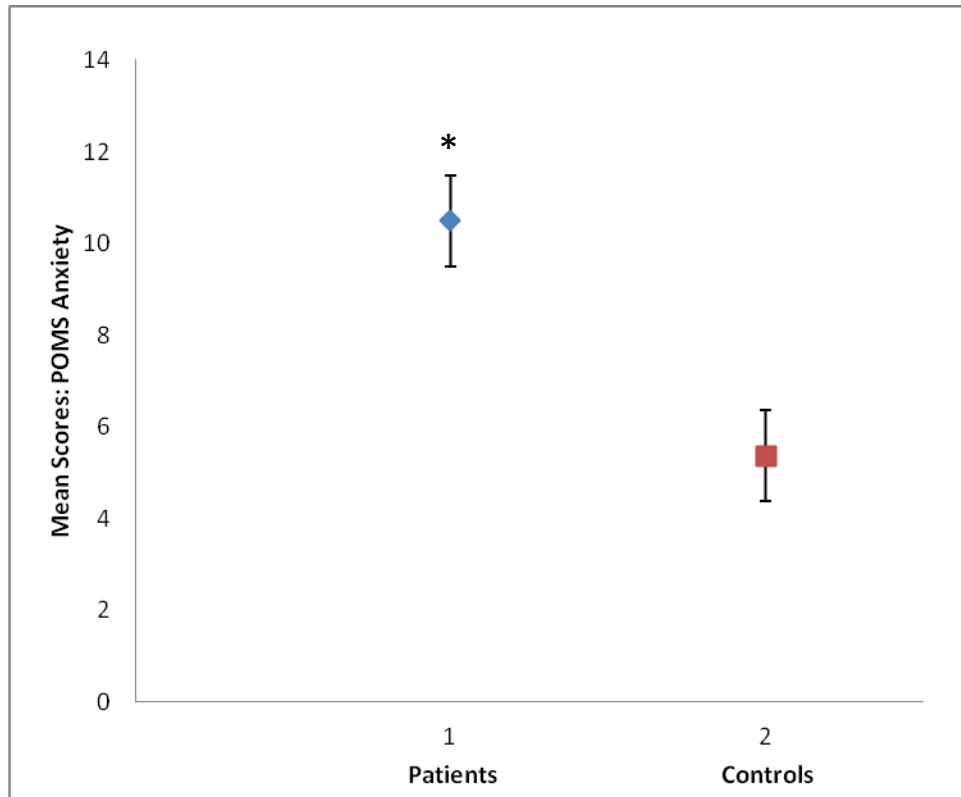


Figure 4: Wellness Variable Anxiety: Main Effect of Group Collapsed across Time 1, 2, & 3



Imaging Data

All coordinates are reported in MNI space. Independent sample t-tests were performed for the working memory contrast of both tasks: 1) Patients T1 versus Controls T1 (exception as previously stated, LNB only); 2) Patients T2 versus Controls T2; and 3) Patients T3 versus Controls T3. Next, errors of omission at T3, mood and anxiety were added to all independent t-tests as covariates. Within group differences were also analyzed using t-tests for both Patients and Controls: 1) Patients T1-T2; 2) Patients T1-T3; 3) Patients T2-T3; 4) Controls T1-T2; 5) Controls T1-T3; and 6) Controls T2-T3. Multiple Regression analyses of mood, anxiety and fatigue were conducted for all within group contrasts stated.

Between Groups- Letter N Back (Working Memory Contrast)

Independent sample t-tests, while controlling for significant differences in errors of omission for the Letter N Back task working memory contrast, revealed that patients showed more activation than controls at both T1 (Table 2) and T2 (Table 3), but not at T3. ROI analyses at T1 revealed that patients activated more than controls in the following region: right inferior temporal gyrus (Figure 5) and at T2 in: Right (R) and Left (L) insula, R superior temporal gyrus, R superior frontal gyrus, L post-central gyrus, R supplementary motor area, and L middle temporal gyrus (Figure 6).

Activation in ROIs were no longer observed in patients versus controls at T2 controlling for mood, but remained significant in the inferior temporal gyrus at T1 (Table 4), plus showed additional activity in the cerebellum (Figure 5). Using anxiety as the covariate, ROIs remained significant at T1 plus showed additional areas of increased activation for patients compared to controls (Table 5) in the: R insula, R superior frontal gyrus and the R supplementary motor area (Figure 5). However, T2 showed a decrease in the number of ROIs previously observed (Table

6) compared to the same contrast without the addition of anxiety as a covariate (Figure 6).

Between Groups- Visuospatial N Back (Working Memory Contrast)

Independent sample t-tests, while controlling for significant differences in errors of omission for the Visuospatial N Back task working memory contrast, showed no significant differences between groups at T2 or T3. However, when mood scores were added as a covariate, patients showed greater activity than controls at T3 (Figure 7) in the L superior frontal gyrus. No significant group differences were found when adding anxiety as a covariate at any of the three time points.

Within Patient Groups- Letter N Back (Working Memory Contrast)

T-tests were conducted, controlling for errors of omission, and revealed that patients showed more activation for the working memory contrast at T1 compared to T3 (Table 7). Similar findings were observed for T2 compared to T3 (Table 8).

ROI analyses demonstrated greater activity at T1 compared to T3 in the Cerebellum (Figure 8) and at T2 compared to T3 in the R Cerebellum and L superior parietal gyrus (Figure 8). Additionally, multiple regressions revealed no significant relationship between neural activity and mood and/or anxiety. However, as fatigue scores increased for patients, a significant increase in neural activity was observed in the supplementary motor area/Brodmann Area 6 (Figure 9).

Within Control Groups- Letter N Back (Working Memory Contrast)

Controls showed significant within group differences at T1 compared to T2 (Table 9). No other significant differences were observed. ROI analyses revealed greater activity for T1 in the following areas: R superior frontal gyrus and the L middle frontal gyrus.

Within Patient Groups- Visuospatial N Back (Working Memory Contrast)

Patients showed significant within group differences at T1 compared to T2. No other significant differences were observed. ROI analyses revealed greater activation at T1 than T2 (Figure 10) in the R supplementary motor area.

Within Control Groups- Visuospatial N Back (Working Memory Contrast)

No significant within group differences were detected for controls on this task.

Table 2: BC Patients > Controls LNB imaging data at T1 for the working memory contrast.

Results presented are Z scores for the maximally activated voxel of the significant areas of activation (L= left, R= right).

*** = $p < 0.05$

Region	MNI Coordinates			z-score	Corr P Value	Cluster K
	X	Y	Z			
Temporal Lobe						
1. R Inferior Temporal Gyrus	50	-46	-18	4.16***	0.001	8134
2. R Superior Temporal Gyrus	46	-18	8	4.06***	0.001	
Sub-Cortical Region						
3. R Thalamus	10	-10	-4	3.73***	0.001	

Table 3: BC Patients > Controls LNB imaging data at T2 for the working memory contrast.

Results presented are Z scores for the maximally activated voxel of the significant areas of activation.

*** = $p < 0.05$

Region	MNI Coordinates			z-score	Corr P Value	Cluster K
	X	Y	Z			
Frontal Lobe						
1. R Insula	40	-30	-20	3.79***	0.001	10632
2. R Superior Frontal Gyrus	16	8	64	3.78***	0.001	12296
3. L Insula	-42	-2	-6	3.21***	0.001	9873
4. R Supplementary Motor Area	12	2	68	3.50***	0.001	12296
Temporal Lobe						
5. R Superior Temporal Gyrus	60	-14	-4	3.66***	0.001	10632
6. R Superior Temporal Gyrus	46	-28	4	3.46***	0.001	10632
7. L Middle Temporal Gyrus	-46	-30	0	3.49***	0.001	9873
Parietal Lobe						
8. L Post-Central Gyrus	-20	-30	56	3.52***	0.001	12296

Table 4: BC Patients > Controls LNB imaging data at T1 for the working memory contrast including mood as a covariate. Results presented are Z scores for the maximally activated voxel of the significant areas of activation.

*** = $p < 0.05$

Region	MNI Coordinates			z-score	Corr P Value	Cluster K
	X	Y	Z			
Temporal Lobe						
1. R Inferior Temporal Gyrus	56	-44	-10	4.29***	0.001	10513
Cerebellum						
2. R Cerebellum	8	-58	-6	3.75***	0.001	10513

Table 5: BC Patients > Controls LNB imaging data at T1 for the working memory contrast including anxiety as a covariate. Results presented are Z scores for the maximally activated voxel of the significant areas of activation.

*** = $p < 0.05$

Region	MNI Coordinates			z-score	Corr P Value	Cluster K
	X	Y	Z			
Temporal Lobe						
1. R Inferior Temporal Gyrus	56	-44	-10	4.36***	0.001	10001
Frontal Lobe						
2. R Insula	38	10	6	4.05***	0.001	10001
3. R Superior Frontal Gyrus	26	40	46	3.33***	0.001	2733
4. R Supplementary Motor Area	16	16	62	3.57***	0.001	2733

Table 6: BC Patients > Controls LNB imaging data at T2 for the working memory contrast including anxiety as a covariate. Results presented are Z scores for the maximally activated voxel of the significant areas of activation.

*** = $p < 0.05$

Region	MNI Coordinates			z-score	Corr P Value	Cluster K
	X	Y	Z			
Temporal Lobe						
1. R Superior Temporal Gyrus	48	-28	0	3.35***	0.001	6822
Frontal Lobe						
2. R Insula	40	-30	20	3.30***	0.001	6822

Table 7: BC Patients T1 > T3 within group comparison LNB imaging data for the working memory contrast. Results presented are Z scores for the maximally activated voxel of the significant areas of activation.

*** = $p < 0.05$

Region	MNI Coordinates			z-score	Corr P Value	Cluster K
	X	Y	Z			
Cerebellum						
1. Vermis	4	-58	-6	3.59***	0.001	10783
2. R Cerebellum	28	-46	-48	3.28***	0.001	10783

Table 8: BC Patients T2 > T3 within group comparison LNB imaging data for the working memory contrast. Results presented are Z scores for the maximally activated voxel of the significant areas of activation.

*** = $p < 0.05$

Region	MNI Coordinates			z-score	Corr P Value	Cluster K
	X	Y	Z			
Cerebellum						
1. Vermis	2	-62	-6	3.37***	0.001	15323
Parietal Lobe						
2. L Superior Parietal Lobule	-28	-50	60	3.26***	0.001	15323

Table 9: Controls T1 > T2 within group comparison LNB imaging data for the working memory contrast. Results presented are Z scores for the maximally activated voxel of the significant areas of activation.

*** = $p < 0.05$

Region	MNI Coordinates			z-score	Corr P Value	Cluster K
	X	Y	Z			
Frontal Lobe						
1. R Superior Frontal Gyrus	24	28	30	3.19***	0.001	5265
2. L Middle Frontal Gyrus	-20	28	34	3.28***	0.001	5265

Figure 5: Image 1 represents the most significant difference for the LNB task where BC Patients have significantly more activity than Controls at T1. Blue crosshairs are centered at the R inferior temporal gyrus ($x = 50, y = -46, z = -18$). Image 2 represents the same contrast with mood as a covariate. Blue crosshairs are centered at the significantly increased difference in cerebellar activity ($x = 8, y = -58, z = -6$) for patients compared to controls. Image 3 represents the same contrast as Image 1 with anxiety as a covariate. Blue crosshairs are centered at the significantly increased difference in activity in the R inferior temporal gyrus activity ($x = 58, y = -44, z = 10$) for patients compared to controls.

A) sagittal view B) axial view

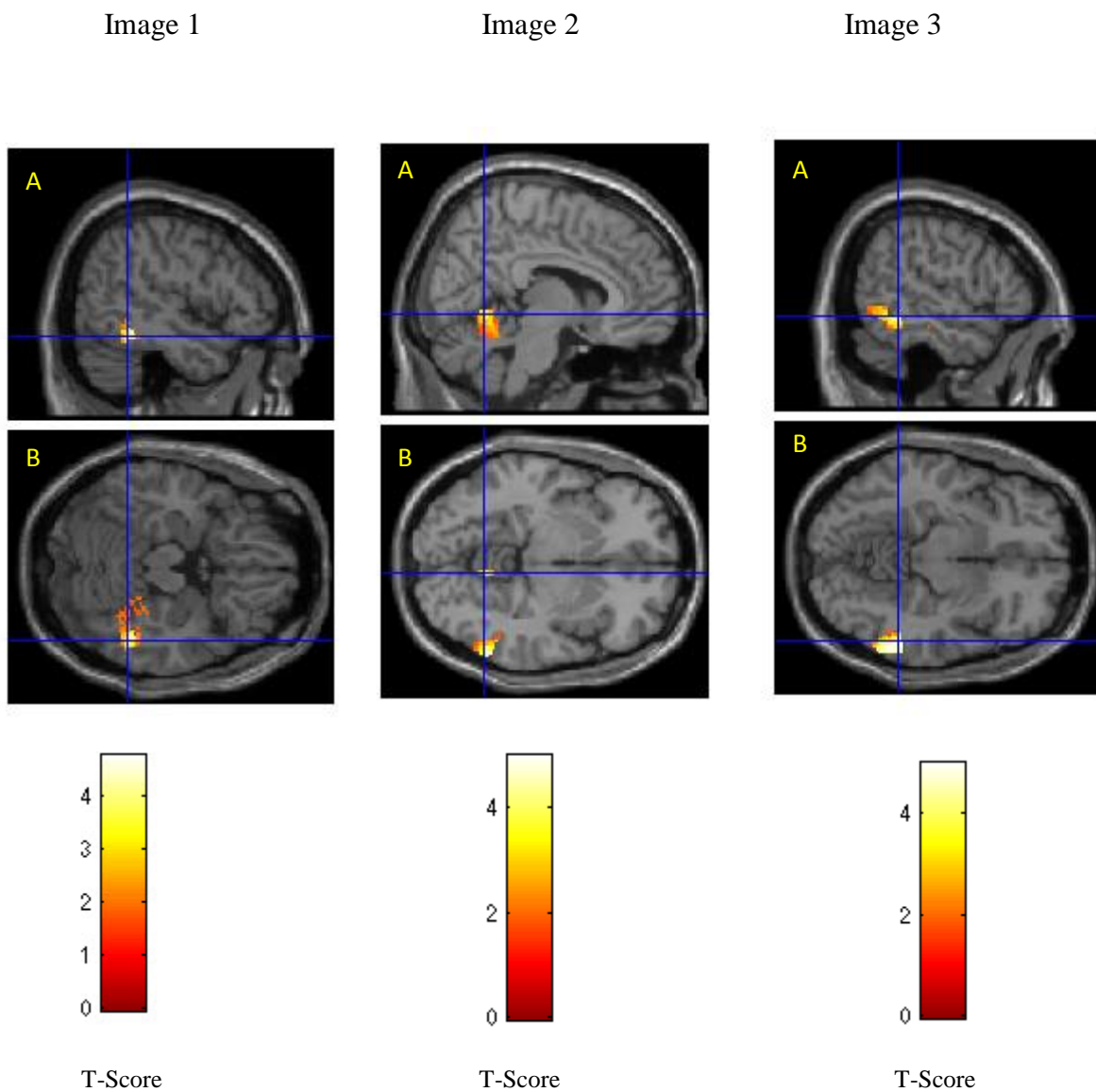


Figure 6: Image 1 represents the most significant difference for the LNB task where BC Patients have significantly more activity than Controls at T2. Blue crosshairs on A and B are centered at the R insula ($x = 40, y = -30, z = 20$), and for C and D at the R superior frontal gyrus ($x = 16, y = 8, z = 64$). Image 2 also represents the same contrast with anxiety as a covariate. Blue crosshairs are centered at the most significant area of activation in the R superior temporal gyrus ($x = 48, y = -28, z = 0$).

A and C) sagittal view; B and D) axial view

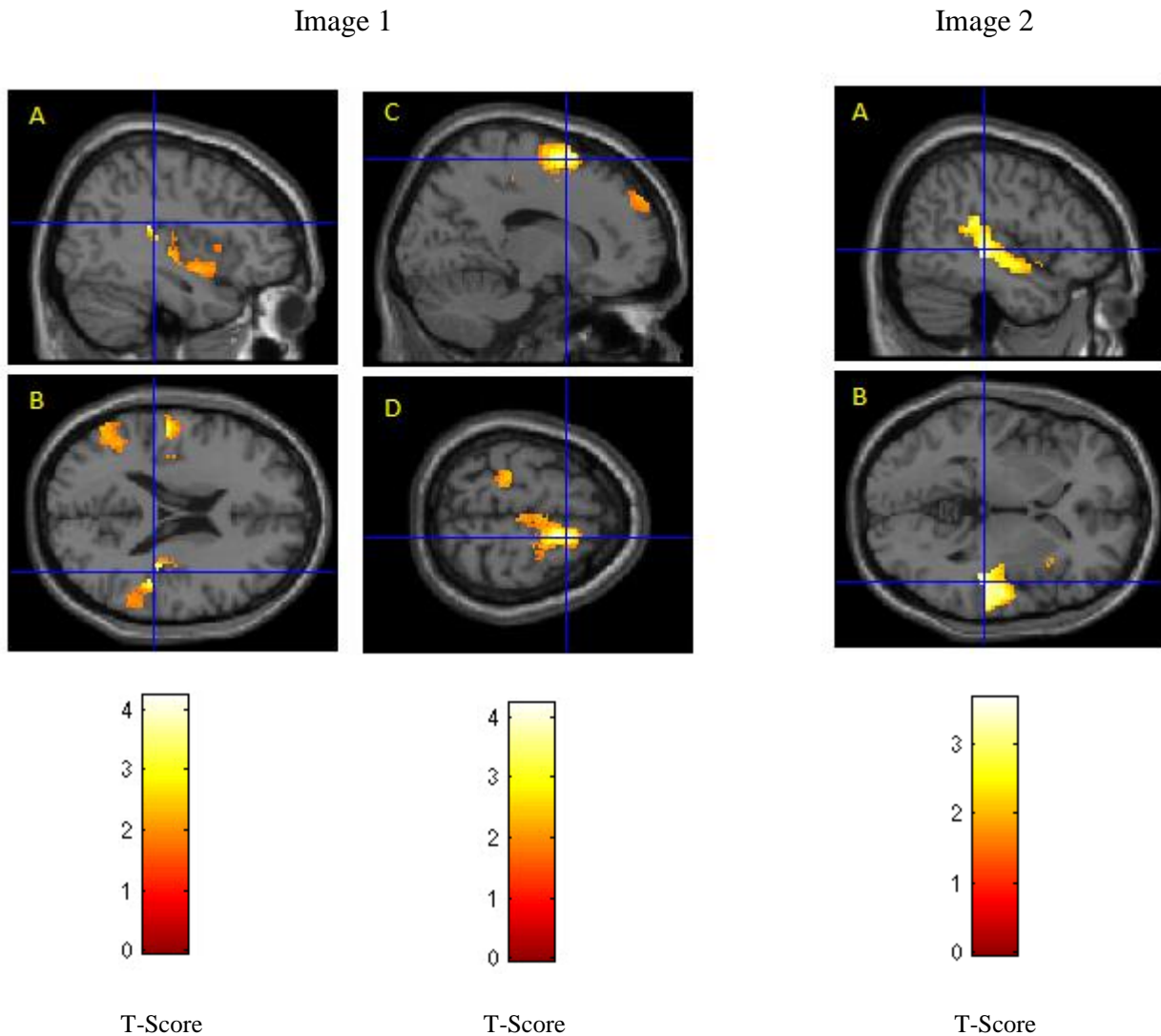


Figure 7: Images represent the most significant difference for the VSNB task with mood as a covariate where BC Patients have significantly more activity than Controls at T3. Blue crosshairs are centered at the L superior frontal gyrus ($x = -18, y = 48, z = 42$, z score of 3.02, $p=.001$).

A) sagittal view B) coronal view C) axial view

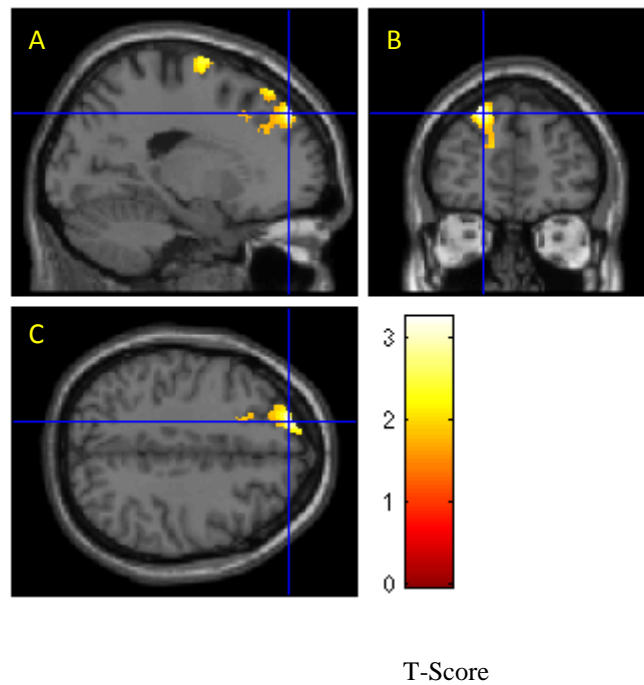


Figure 8: Image 1 represents the most significant difference for the LNB working memory contrast where BC Patients have significantly more activity at T1 than T3. Blue crosshairs are centered at the R vermis of the cerebellum ($x = 4, y = -58, z = -6$). Image 2 represents the most significant difference for the same contrast where Breast Cancer Patients have significantly more activity at T2 than T3. The blue crosshairs are centered at the R vermis of the cerebellum ($x = 2, y = -62, z = -6$).

A) sagittal view B) axial view

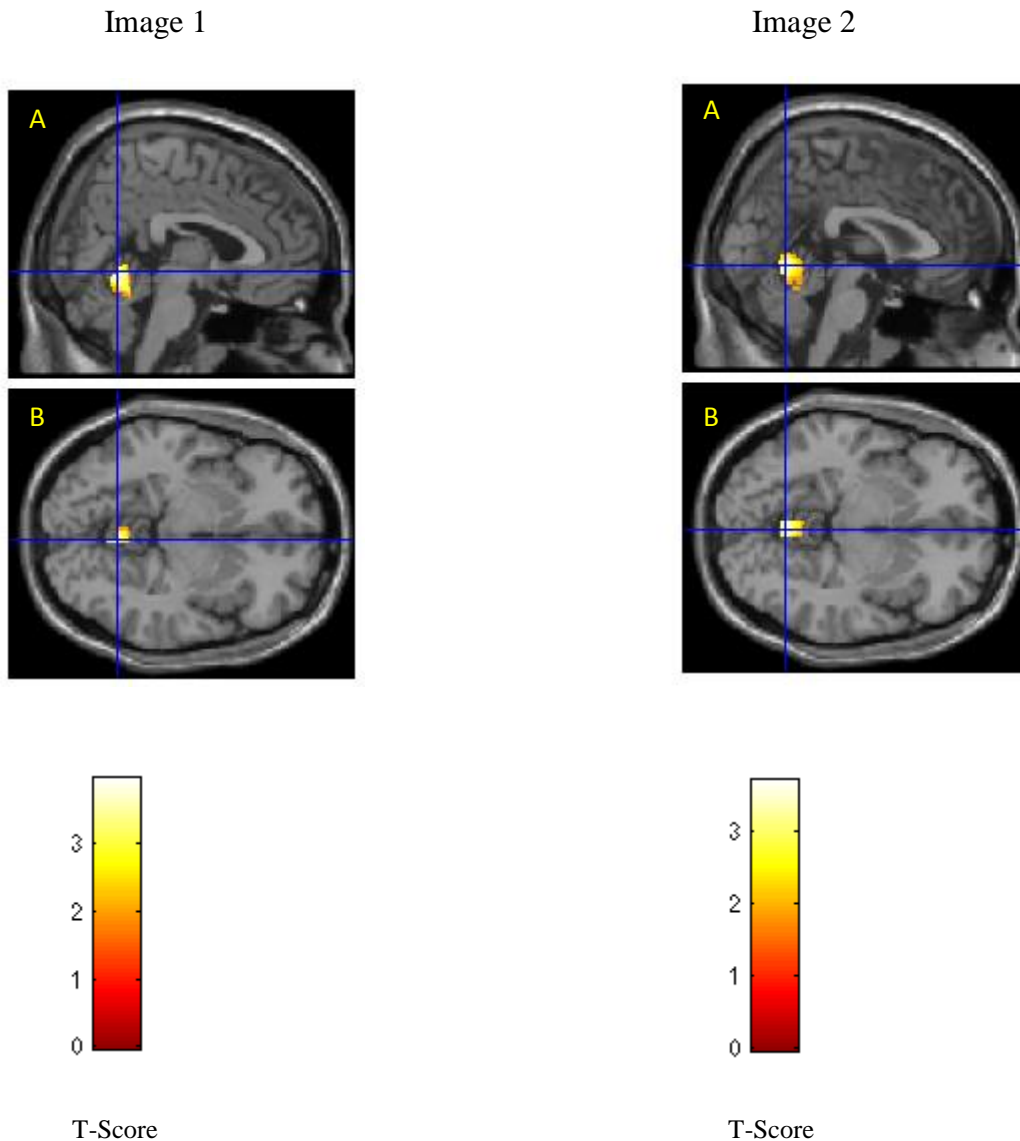


Figure 9: Images represents multiple regression data (T1, T2, T3) for the LNB working memory contrast for BC Patients using fatigue as a variable of interest. Blue crosshairs are centered at the increased activity observed in the L supplementary motor area as fatigue scores increased for BC patients ($x = -2, y = -8, z = 56, z$ score of 3.87, $p=.001$).

A) sagittal view B) coronal view C) axial view

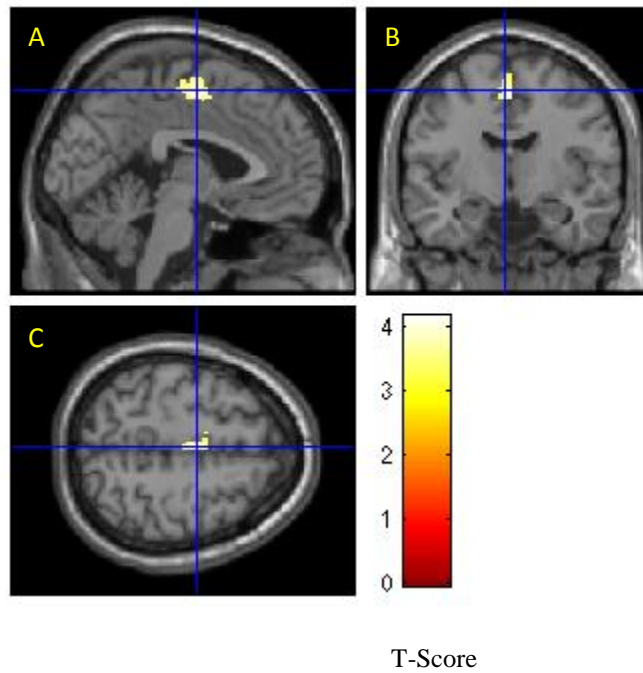
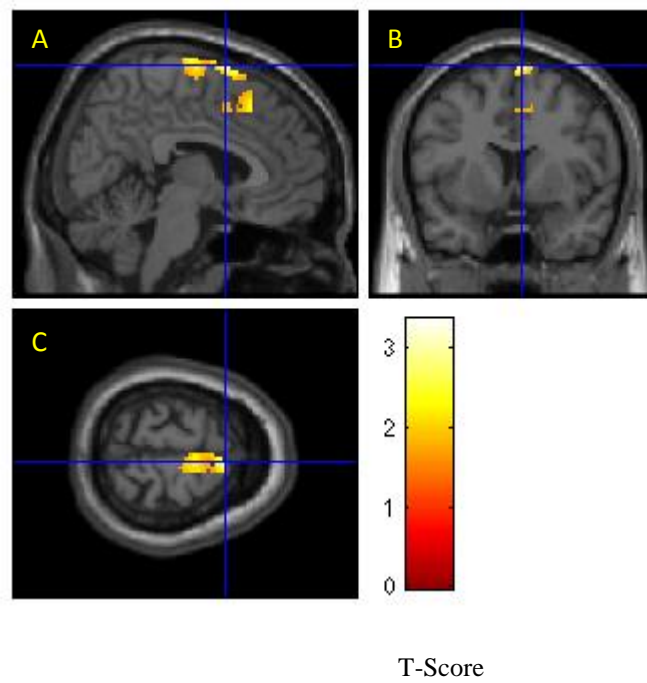


Figure 10: Images represent the most significant difference for the VSNB working memory contrast where BC Patients have significantly more activity at T1 than T2. Blue crosshairs are centered at the most significant increased activity in the R supplementary motor area ($x = 6, y = 8, z = 74$, z score of 3.36, $p=.001$).

A) sagittal view B) coronal view C) axial view



Discussion

This fMRI study provides support for a significant impact of mood, anxiety, fatigue and chemotherapy on neural processing during working memory, in a group of women with BC. The contribution of each of these variables was not possible to quantify, highlighting the complexity of determining the neural correlates and the mechanisms that underline chemo fog.

There were significant differences in neural activity between BC patients and matched control participants during working memory tasks before and shortly after chemotherapy. At one year post chemotherapy, these differences were no longer observed for one of the working memory tasks. Both mood and anxiety were found to be moderators in the observed findings. Additionally, a positive correlation was revealed between fatigue scores and neural activity for the BC group during working memory.

Pre Chemotherapy: BC Patients Compared to Controls (LNB Working Memory Task)

Results observed for the LNB working memory task prior to chemotherapy intervention support earlier findings of pre chemotherapy activation differences between women with and without BC (Cimprich et al., 2010; Scherling et al., 2011; McDonald et al., 2012). The most significant results observed for this study after surgery but before chemotherapy revealed greater neural activity for BC patients compared to control participants in the right inferior temporal gyrus. This region is involved in higher order processing of visual information and for the temporary storage of speech-based information. In Baddeley's working memory model (1986), this component makes up part of the phonological loop. The phonological loop is a subsystem under the control of the central executive. Thus, while speech based information of the LNB working memory task is temporarily stored within the phonological loop, it is the central

executive that is making all the decisions about what data to attend to, select, and manipulate in that subsystem. Research has indicated parietal and temporal lobe involvement during activation of the phonological loop (Paulesu et al., 1993; Smith & Jonides, 1997), whereas the frontal lobes are correlated with executive processing (Owen et al., 2005).

Applying the construct of Baddeley's working memory to our findings, increased neural activation observed in the right inferior temporal gyrus for BC patients compared to controls to complete the same working memory task suggests greater demands on the phonological loop circuitry of BC patients even before the introduction of chemotherapy. Analysis of performance data for this LNB working memory contrast indicated that both groups were successfully performing the task with no significant differences between the BC patients and the control group in their reaction time or in the number of errors made (commission/omission). The N Back working memory task has been well researched demonstrating that for all participants (including controls) once the working memory load has been increased, there is an increase in neural activity (Braver et al., 1997; Manoach, D. et al., 1997). Together this suggests that the BC patients had to work harder to accomplish the task by increasing blood flow in the temporal lobe. These results and those of others previously mentioned (Cimprich et al., 2010; Scherling et al., 2011; McDonald et al., 2012) also support the hypothesis that BC patients are vulnerable to changes in neural activity even prior to chemotherapy. This implies that other variables, in addition to chemotherapy, may be playing a role in the cognitive decline observed post chemotherapy such as the disease itself, stress, and/or mood disturbances.

Post Chemotherapy: BC Patients Compared to Controls (LNB Working Memory Task)

One of the most common cognitive complaints of BC patients after chemotherapy is a working memory problem (Ganz, P. et al, 2013). Our imaging findings showed additional areas of greater activation for the LNB working memory task approximately one month post chemotherapy intervention versus pre chemotherapy for the BC group compared to the control group. This increased neural activity strongly supports the hypothesis that chemotherapy is also having an effect in working memory for the BC patients. Results provide empirical evidence for further alterations in patterns of neural activity post chemotherapy with BC patients having to work harder than control participants to perform the same working memory task (Braver et al., 1997; Manoach, D. et al., 1997). The most significant results post chemotherapy were observed in the following areas: R&L Insula, R superior and L middle temporal gyri, R superior frontal gyrus and the L post-central gyrus. This latter structure is located in the parietal lobe and houses the primary somatosensory cortex, an area responsible for the reception of touch. Significant activity observed in the L post central gyrus for those in the BC group might suggest greater awareness of the response pad and its importance in performing the task. Increased activation in the left hemisphere would be expected due to right handed responding.

The insula is located beneath the temporal and frontal lobes and has a number of functions, one of which is related to cognition. More specifically, it appears to play a role in decision making and in particular, showing larger activation with more risky responses (Paulus et al., 2003). Given the Baddeley working memory model, perhaps greater bilateral activation in the insula denotes that the central executive is having to work harder in its determination of what to do with the contents of the phonological loop, requiring increased blood flow to direct attention appropriately. As highlighted in the Cimprich study (2010), BC patients have shown

bilateral brain activation in high-demand task conditions (in both selective attention and working memory tasks) with recruitment of additional components of attention/working memory circuitry compared to healthy control participants. In addition to the increased bilateral insula activity, we observed significantly more activation in both the R superior and L middle temporal gyri of BC patients, again areas known to be associated with Baddeley's phonological loop. Equally important, activation differences were observed in the superior frontal gyrus, with a number of functional studies demonstrating that the superior frontal gyrus is activated in working memory paradigms (Courtney et al., 1998; Postle et al., 2000; Rowe et al., 2000; Johnsons et al., 2003). In short, our findings demonstrate that when executive functioning occurs, including that related to working memory, multiple neural systems are involved. In particular, our results imply there is an increased demand on both the phonological loop and the central executive of BC patients, demonstrating that chemotherapy is having a significant effect on cognitive processing.

No significant differences were observed between BC patients and control participants at the third imaging session, one year post chemotherapy for the LNB task. BC patients made significantly more errors of omission than the control group one year post chemotherapy intervention. This, however, did not have a significant impact on neural activation, nor did mood or anxiety. The fact that neural differences are no longer observed one year later is encouraging for the BC group. The truth is that we were happy to see this but are not sure that it is representative of the BC population. It might well reflect a sample bias as these patients were very dedicated to the study. This was also the case for the controls as the BC patients nominated their own controls and thus the controls were invested in the study as they progressed through the treatment process with their friend or relative. It is possible that the BC patient group was comprised of individuals who were less vulnerable to the effects of their disease compared to

other BC patients which enabled them to volunteer, for example fewer negative effects of surgery, including mood and anxiety. Furthermore, fMRI results represent group analyses rather than individual participant results. This is a limitation of this type of research given that the groups cannot be broken down into smaller groups (affected compared with non affected) due to power issues.

Role of Mood and Anxiety: BC Patients compared to Controls (LNB Working Memory task)

It has been shown that depression and anxiety are two of the most common comorbidities for BC patients (Frick, Tyroller, & Panzer, 2007). Our results are significantly altered when accounting for the contribution that mood and anxiety play in neural activity pre and right after chemotherapy. Neural activation of the cerebellum was shown to be suppressed by mood in BC patients pre chemotherapy. This was observed by using mood as a covariate in the imaging analysis. The cerebellum, or ‘little brain,’ accounts only for ten percent of the entire brain mass, but it contains as many neurons as all of the rest of the CNS combined (Nolte, 2009). The cerebellum appears to play a major role in different brain functions other than balance and motor control, including emotional regulation and cognition. One theory suggests that the cognitive functions of the cerebellum allows for increased ability in speed, efficiency and adaptability of original cerebral functions, including that of working memory (Vandervert, L., & Liu, P., 2007). These authors suggest that in the same manner that cerebellar models account for the control of repetitive bodily movements (learned and fed back to the cerebral cortex), one can imagine the same type of model linking cerebellum and cognitive processes such as working memory. Indeed, there is literature to support that this link between the cerebellum and the cerebral cortex makes attentional, visuospatial and language functions faster, more efficient and

adaptive (Akshoomoff, et al., 1997, Baddeley & Andrade, 1998). This processing seemed to be suppressed by the mood of the BC patients in our study, however, recovered one year post chemotherapy.

The impact of mood post chemotherapy was also significant as it appears to have accounted for the group differences. No neural differences were observed between groups immediately following chemotherapy when controlling for mood. We hypothesize that by the time chemotherapy cycles have finished, mood disturbances have increased since the time of diagnosis, and indeed may have been the main contributor behind any existing differences seen post chemotherapy without the use of mood as a covariate. However, the effects of chemotherapy intervention cannot be ruled out. It is entirely possible that neural activity was changed by the chemotherapy resulting in poor mood scores for the BC group, versus the notion that mood itself changed the neural activation. The root mechanism is unclear and needs further investigation.

High levels of stress have also been associated with working memory challenges (Gass & Curiel, 2011). Investigating the role of anxiety for the LNB working memory contrast between groups before and after chemotherapy provided a more comprehensive account of our findings. It follows that once a patient is diagnosed with BC, anxiety levels will increase (Lim et al., 2011). Moreover, several studies have provided evidence of a positive correlation between increased anxiety scores and complaints of cognitive difficulties (Shilling et al., 2005; Bender et al., 2006; Jenkins et al., 2006; Monk et al., 2008). By controlling for anxiety (removing its influence on the results) before chemotherapy in this study, we determined that anxiety was in fact contributing significantly to the group differences observed. Anxiety was suppressing activation differences between groups in the R insula, R superior frontal gyrus and R supplementary motor area. The

latter is located in the superior frontal gyrus, anterior to the primary motor cortex, and is very important for the planning of movements. Given previous discussion on the importance of both the insula and the superior frontal gyrus in working memory, this overall increased neural activation indicates high anxiety levels of the BC group were suppressing initially observed functional activity.

Comparing the individual contributions of mood and anxiety, results pre chemotherapy are suggesting that anxiety was playing a larger role than mood for this task due to the number of areas showing greater activation when controlling for anxiety. This seems reasonable given that at the beginning of their BC journey, patients are facing a great deal of uncertainty about the impact of their diagnosis. Furthermore, post chemotherapy results for this same working memory contrast (keeping anxiety as a covariate) showed a reduction in neural activity. Essentially, some but not all of the areas previously shown to be significant (without the use of covariates) remained significant post chemotherapy. This implies anxiety partially explained neural activation of the BC group post chemotherapy, bearing in mind that mood scores accounted for all significant differences post chemotherapy between groups for this same working memory contrast. It would appear that the longer the BC patient is exposed to the impact of being diagnosed with cancer, the more likely their mood will be negatively affected, but anxiety levels will decrease by comparison once the chemotherapy is complete. The patients' POMS scores over time also support this suggestion.

Overall, in comparing BC patients to control participants for the LNB working memory task, findings indicate that chemotherapy, anxiety and mood all significantly affect neural activity during working memory for the BC group before and right after chemotherapy, but not one year following chemotherapy. This indicates a gradual reduction of any previous differences

observed in neural activity between BC patients and control participants. Our results can be considered optimistic for the BC group given that one year post chemotherapy, mood and anxiety did not have an impact on neural functioning during working memory that can be observed with fMRI, nor did the chemotherapy.

McDonald and colleagues (2012) recently published similar findings. Using fMRI, these authors examined working memory in the BC population employing an auditory N Back task (including a 3 Back condition) and performance was assessed roughly on the same timeline as in our study including a cognitive assessment pre chemotherapy. Their results showed that at baseline (post surgery, pre treatment) BC patients demonstrated increased bi-frontal and decreased left parietal activation compared with a control group. One month post chemotherapy, findings showed decreased frontal hyperactivation for BC participants compared with control participants. However, one year later performance was restored to near that observed at baseline. It was also concluded that factors such as mood, anxiety and fatigue more than likely did not play a role in their findings due to the lack of between group differences on such measures.

This was not the case in this thesis as mood and anxiety were significant modulators of neural activity during working memory. There is a discrepancy in the literature generally on these measures, and given the differences just cited between this study and the McDonald study (2012), it is important for future studies to consider the inclusion of biomarkers when possible. For example, cortisol samples could be collected and analyzed as a more objective measure of anxiety/stress compared to subjective self-reported measures. Cortisol, made in the adrenal glands, is a hormone directly related to human stress, and research has shown elevated cortisol levels to be associated with smaller hippocampal volumes (Pruessner et al., 2005; McEwen, B., 1999). This is an area of the brain not only connected to the prefrontal cortex, but closely linked

to memory. Notably, Lupien et al., (1999) reported significant effects of induced hydrocortisone (a drug similar to natural cortisol) on working memory. Considering the research, future investigations might consider the inclusion of salivary cortisol measurements based on research that has demonstrated measurements of saliva cortisol during morning hours is a good substitute for measurement of cortisol found in serum or plasma (Ljubijankic et al., 2008).

Additionally, the McDonald study recruited BC participants who were treated with chemotherapy, and those without, along with healthy control participants. Independently comparing the two groups of BC patients to the control group post chemotherapy intervention, both BC groups showed decreased frontal hyperactivations. Combined with the result of compromised cognitive functioning pre chemotherapy, this finding helps explain the growing interest in other factors that might be relevant to the concept of chemo fog such as the role of the cancer itself. Both the McDonald's findings and our own suggest that cognitive impairment observed in BC patients cannot be attributed to chemotherapy alone.

Pre and Post Chemotherapy: BC Patients Compared to Controls (VSNB Working Memory task)

According to Baddeley's theory of working memory (1986), the component responsible for the manipulation and temporary storage of visual and spatial information essential to the VSNB working memory task is the visuospatial sketchpad. Stimuli for this task are processed in what is known as the ventral (object recognition) and dorsal (how/where) pathways. The ventral pathway processes information around object identification in the temporal lobe, whereas the dorsal pathway processes spatial location in the parietal lobe. Identical to the phonological loop, the visuospatial sketchpad is a passive slave system under the control of the central executive. Thus, while a completely different system is being activated during this working memory task

compared to the LNB task, it is interesting that the findings for the VSNB working memory task were not as robust. To clarify, no analyses were performed for the VSNB working memory contrast pre chemotherapy intervention as these results were previously documented (Scherling et al., 2011). A brief summary of reported findings include no significant group differences observed in neuropsychological tests, estrogen, or cortisol, but significant differences were observed for the imaging data. Results varied depending on the type of statistical analysis performed, and while both mood and anxiety scores were significantly different between groups, only mood was observed to modulate neural activity. Specifically, this study demonstrated greater neural activity pre chemotherapy in several areas for BC patients compared to controls during a Visuospatial working memory task including: inferior frontal gyrus, insula, thalamus and, midbrain. Reaction times modified findings observed, removing right midbrain differences between groups but also accounting for an increase in bilateral thalamic activations in patients compared to controls. Beyond the differences observed in neural activations when accounting for mood, days since surgery as well as errors of commission also altered neural activity when considered as a covariate. Taken together, all baseline findings provide a strong rationale to consider additional variables of interest beyond chemotherapy itself.

Analyses performed for this thesis found no significant differences between BC patients and controls post chemotherapy, or one year later for the VSNB working memory task. Furthermore, there were no significant differences between groups at either time point when controlling for anxiety. However, in contrast, when controlling for mood, significant differences were observed one year post chemotherapy. BC patients showed greater activation than controls in the L superior frontal gyrus. It appears that more depressed mood states were suppressing neural activity in this region. Given that this area of activation fits with Baddeley's working

memory model, we have possible empirical evidence supporting complaints about the diminished working memory of BC patients. It is difficult to interpret the overall results due to no significant differences observed immediately following chemotherapy. Perhaps the chemotherapy had a slow acting impact and it took months for the outcome to be observed. Again, future research, perhaps with DTI, are required to investigate the mechanisms of action of chemotherapy.

Also interesting is the comparison of findings between the two working memory tasks. Although not significant, the performance data for both working memory tasks revealed longer response times as well as fewer correct responses for the BC group compared to the control group. Even errors of omission, which were significant at the last imaging session, showing BC patients making more errors of omission than control participants, were observed for both working memory tasks. However, when errors of omission were controlled for within the imaging data one year post chemotherapy, observed neural activations remained the same, thus indicating they did not have an impact on functional activity. One possible explanation for the neural differences observed between the two working tasks may be that members from both groups often reported that the VSNB working memory task was the more challenging task. Perhaps the VSNB working memory task did not reflect overall differences between groups due to the reported spatial manipulation difficulties of most participants. It was also administered last in the battery of fMRI tasks and thus fatigue may have played a significant role in the results.

Within Group Differences (Both tasks)

Similarly, findings for the within group analyses were less revealing for the VSNB working memory task compared to the LNB working memory task. The most significant within

group differences were observed for the BC group during performance of the LNB working memory task. Comparing imaging data from patients at all time points demonstrated greater activity for BC patients in the cerebellum before and immediately following chemotherapy and in the L superior parietal gyrus right after chemotherapy compared to imaging data one year post chemotherapy.

Also, as fatigue scores for the BC patients increased, there was an increase in neural activation in the L supplementary motor area for the LNB working memory task. Our decision to explore the role of fatigue within the BC group was based on previous cancer literature. For example, research has shown that fatigue is a symptom often reported by those with cancer as well as by those who are receiving chemotherapy treatment (Mitchell, 2010; Spichiger et al., 2011). It has also been reported that at diagnosis more than 30% of BC patients report symptoms of fatigue (Bower et al., 2000). Thus, it has been postulated that the BC patient's working memory may be vulnerable to the effects of fatigue prior to treatment (Cimprich et al., 1992, 1998). Yet, it is not uncommon for imaging studies to report non-significant differences between groups on this measure and thus exclude fatigue from further investigation. For example, the McDonald study (2012) reported mean fatigue values calculated from a self-report measure that were slightly elevated, but this applied to all three participant groups (BC patients who received chemotherapy, BC patients who did not, and a healthy control group). Recent work by our own lab (Scherling et al., 2011) that used biological measures (patient blood work) to assess fatigue in BC patients prior to chemotherapy observed average values that were in the normal range. Setting aside between group differences, a goal for this thesis was to further explore the effects of fatigue in BC patients. Other cancer studies have noted a link between fatigue and self-reported memory/attention difficulties (Biegler et al., 2009). Similarly, 60-96% of BC patients

report increased fatigue during treatment (Wagner et al., 2004) and evidence showing chronic fatigue can continue to exist for years post diagnosis (Bower et al., 2006; Servaes, P., et al., 2002).

These results warranted investigation of the impact of fatigue on working memory activation patterns in the BC patients using the Functional Assessment of Cancer Therapy-Fatigue scale (FACT-F). The FACT-F has demonstrated good reliability ($r = .87$) and has been found to successfully discriminate patients based on hemoglobin levels (Cella, D., 1997). Given our findings of significantly more activity in the supplementary motor area, an area key for the planning of complex movements, one can see fMRI allows for unique opportunities to see through a window into the working brain. Future work might consider between group analyses using a different measure of fatigue. Indeed, work recently published by our lab found fatigue accounted for some of the differences in brain activation observed both before and after chemotherapy between BC patients and controls using a verbal memory retrieval task (Lopez Zunini et al., 2012).

The only within group difference observed for the VSNB working memory task was for the BC group. Findings again showed greater activation for BC patients in the supplementary motor area pre chemotherapy compared to post chemotherapy, but this was observed in the R hemisphere. This might suggest less effort was required post chemotherapy due to the experience gained at the first imaging session. Results from the LNB showed a positive relationship between fatigue and activity in the supplementary motor area so perhaps this result was related to fatigue. This was not determined with the present data.

It is worth noting that the only significant finding for the control group in this entire thesis was a within group difference during the performance of the LNB working memory task. Control participants showed greater activation at the first imaging session compared to the second imaging session with significantly more activation observed in the R superior frontal gyrus and the L middle frontal gyrus. Activity in the middle frontal gyrus has been associated with motor planning as well as complex non-motor tasks such as decision making, discrimination, computation, and reasoning (Erdler et al, 2000; Tanji & Mushiake, 1996). This decrease in neural activity at the second imaging session may be explained by practice effects. Initial exposure to the task could have required more effort, but by the second time, familiarity likely influenced task performance. Certainly the lack of any significant differences for controls in this task after one year supports this contention.

In conclusion, within group comparisons provided evidence of chemotherapy intervention having an impact on working memory neural processes, but the chemotherapy was not acting alone. Fatigue accounted for some of the neural activity differences observed over time and treatment, and gradual improvement was observed one year post chemotherapy.

Neuropsychological Support

As previously mentioned, this fMRI study was part of a much larger BC study that included the administration of neuropsychological measures. Analyses of the same participants involved in the fMRI component examining four cognitive domains (Verbal Memory, Visual Memory, Working Memory, and Information Processing Speed), revealed poorer performance on an overall cognitive summary score for the BC group post chemotherapy compared to pre chemotherapy using the scores of the control group as the normative sample. The driving force

behind the cognitive decline from the first assessment until the second assessment (matched to fMRI imaging sessions) was processing speed. However, the only significant differences observed between groups in this domain were from pre to post chemotherapy. Matching the fMRI data, there was a gradual but steady improvement from post chemotherapy to the last imaging session one year later.

The recently published neuropsychological results for the entire study sample ($n = 60$), conducting assessments after surgery but prior to commencing chemotherapy, and then again following each chemotherapy cycle, showed significant progressive decline for the BC group compared to the control group in an overall cognitive summary score as well as in working memory (Collins et al., 2012). Future analyses and integration of the two data sets will provide more valuable information in terms of helping researchers, practitioners and BC patients understand this phenomenon known as chemo fog.

Mediating Factors/Limitations

As recognized in our opening remarks, there are limitations to be considered when interpreting the findings of BC research. For example, the dose of chemotherapy administered was not controlled for in this study. Similarly, consideration must be given to the fact that various chemotherapy treatments were accepted. Also, chemotherapy can trigger an immediate cessation of menses in approximately 50% of BC patients exposed to treatment (Lower et al., 1999). Chemotherapy induced menopause may affect cognitive functioning with post menopausal studies revealing decreased bilateral hippocampal volumes for those women reporting declining cognitive abilities (Lord et al., 2008). Research has shown that estrogen receptors are found in brain regions linked to working memory (McEwen, 2002) and that

estrogen therapy (hormonal replacement therapy) may have a positive effect on cognitive functioning. For example, this compound has been found to modulate activity in temporal and frontal regions during cognitive performance (Berman et al., 1997; Joffe et al., 2006).

In the McDonald (2012) study, almost 40% of those in the chemotherapy BC group experienced instant menopause whereas only 25% of those in the non chemotherapy BC group entered menopause (assumed due to natural causes) and just over 13% in the control group. In our study, almost the entire sample of BC patients both pre and peri menopausal experienced instant menopause following chemotherapy. It should be acknowledged that the risk for permanent amenorrhea appears to be lower for women less than 35 years (Lower et al., 1999), and that our study had no participants below this cut-off. This reduction in estrogen levels is likely to have an effect on any results observed considering the association between menopause and a decline in both memory and attention (O'Bryant et al., 2003; Weber et al., 2012). Hormonal effects on cognition become even more complicated with BC treatment regimes that include drugs such as tamoxifen.

Tamoxifen is prescribed as an adjuvant BC treatment for those women who have tested positive for estrogen receptor (ER+) BC. Estrogen has been shown to promote the growth of BC cells in such tumours. Tamoxifen is a drug that blocks the effects of estrogen and is often referred to as an anti-estrogen because of its ability to inhibit the synthesis of estrogen. While often considered necessary in a BC treatment regime, tamoxifen therapy does mean a further reduction in the supply of estrogen for the BC patient. This could have a significant impact on any observed differences in cognitive functioning post chemotherapy.

Previous work by Collins et al. (2009) found two types of hormonal therapies (tamoxifen and anastrozole) to have a negative impact on verbal memory and processing speed in BC patients. At the same time, not all research has supported a link between decreased estrogen levels and complaints of cognitive impairment (Tchen et al, 2003). It was our hope for this thesis to be able to further investigate the role of estrogen on working memory by comparing those women who received tamoxifen to those who did not. Based on previous research, it was anticipated that only half of our sample would require tamoxifen. In reality, 16 of the 19 fMRI BC patients were exposed to tamoxifen therapy post chemotherapy. It is our understanding that BC treatment regimes are now moving toward a more stringent inclusion of this adjuvant hormonal therapy.

In addition, it is important to mention that while structural changes were not the focus of this thesis (and will be published separately), one must consider the relevant literature and how it plays into our understanding of chemo fog. For example, two studies (Cimprich et al., 2010; Vardy et al., 2007) as well as published results of our own lab (Scherling et al., 2012) have reported subtle structural differences in BC patients compared to controls before the introduction of chemotherapy. Perhaps these differences are due to the disease itself, or other factors such as mood and anxiety. Other BC imaging research has revealed structural changes post chemotherapy between BC patients and controls (Deprez et al., 2011; McDonald et al., 2010; Abraham et al., 2008).

It is possible that post chemotherapy neuroanatomical changes result due to the chemotherapy intervention itself, and thus are directly linked to any functional differences observed. To elaborate, antineoplastic agents, routinely used in chemotherapy, are able to pass through the blood brain barrier and affect the brain. Troy et al., (2004) as well as Tuxen &

Werner (1994) have shown higher than normal concentrations of such neurotoxin agents to be present in the brains of chemotherapy treated individuals. Essentially chemotherapy treatment can leave white matter exposed to neurotoxins affecting the integrity of the white matter tracks. Even without establishing a cause/effect relationship, pre-existing differences in brain structure may be contributing to some of the working memory effects observed both pre and post treatment, leaving the patients more vulnerable to the impact of chemotherapy. Certainly, both depression and anxiety have also been linked with structural changes in temporal and frontal regions (Lamar et al., 2012) which can affect memory functions (Lupien et al., 1999; Lupien & McEwen, 1997). Similar to the functional results of this thesis however, observed structural differences tend to recover over time (Inagki et al., 2007; Deprez et al., 2011; Abraham et al., 2008; McDonald, 2010).

In summary, the imaging data observed in this study, although subject to a number of considerations, does provide empirical evidence for the existence of a neural basis for chemo fog in BC patients. It also highlights the importance of study design, methodology and the multifaceted nature of the potential problems investigating this phenomenon.

Conclusion

This study contributes to the BC chemo fog literature but is specific to Stage I and Stage II BC. Our data provide valuable information regarding the phenomenon of chemo fog, demonstrating neural activation differences even before the administration of chemotherapy. Our findings also offer empirical evidence that chemotherapy affects brain activity during working memory, and differences do not appear to exist one year after chemotherapy for certain types of working memory. Furthermore, results show that other key factors such as fatigue, anxiety and

mood are involved in chemo fog. Understanding the nature and the impact of these factors and treatment is important for the thousands of women who suffer from this impairment and for family and friends around them. It is also important to the medical professionals who administer care to BC patients, as they need to be aware of cognitive impairment as a potential side effect of not only chemotherapy but of the disease itself and the associated stressors.

Cognitive impairment referred to as chemo fog does not encompass the multi-factorial nature of the problem. A more accurate term would be cancer related cognitive impairment. Chemotherapy is but one piece of the puzzle as revealed by this thesis and the work of others. Future imaging work should include further investigating brain changes in BC patients prior to chemotherapy intervention (i.e. the role of the disease itself and variables of interest such as surgery, estrogen, cytokines, mood, anxiety and fatigue) and over time. In particular, a between group measure of fatigue over time should be considered. Clearly findings of this thesis and others show that neural activation differences during cognition in BC patients are multifactorial. Yet, additional research is needed to fully understand the extent of chemo fog in BC patients and the neural mechanisms responsible for these problems. If impairment exists, even mild impairment, it may have an impact on quality of life for the BC patient, and/or impact their ability to function both at home and work. Understanding the problem and the etiology can help inform the BC patient and aid in their decision making with respect to the illness. In conclusion, few working memory neural differences between BC patients and control participants one year following chemotherapy intervention is extremely encouraging for women with BC as it may represent recovery.

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Appendix A

Letter N-Back

Press for X

X (press)

C

X (press)

M

X (press)

X (press)

N

Press for 2-back

C

B

G

B (press)

A

N

A (press)

F

F

B

Appendix A

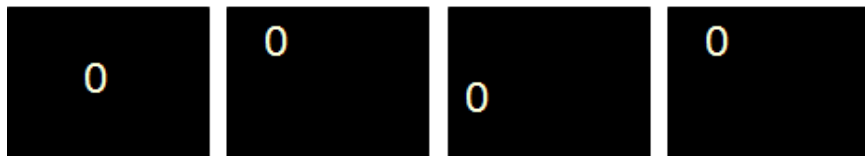
Visuospatial N Back

Match to Centre



Press

Press for 2-back



Press

Appendix B

Neuropsychological Procedure and Measures

A clerk from the medical oncology clinic at TOHRCC reviewed appointment schedules from September, 2008 to April, 2010 to identify all new BC patients meeting preliminary inclusion/exclusion criteria for the study. Upon identifying a potential study candidate, the clerk placed a notice on the front of that patient's chart reminding the oncologist to introduce the study to the candidate. If eligible and agreeable, the patient was then referred on to study personnel for further screening. This study was approved by the Ottawa Hospital Research Ethics Board and informed consent was obtained in all cases.

All participants underwent a baseline assessment lasting approximately 2-1/2 hours that comprised a social and medical history, questionnaires to assess mood, fatigue, and subjective cognitive function, a battery of pencil-and-paper neuropsychological measures and a brief computerized cognitive test battery. In the case of the BC patients, this baseline assessment was done following recovery from surgery but prior to commencement of chemotherapy. Most of the cognitive tests and questionnaires were re-administered to the BC patients following each chemotherapy cycle (in sessions lasting approximately 1-1/2 hours), typically shortly before the next chemotherapy treatment to allow sufficient time for any acute side effects to subside. The assessment schedule for each control participant was matched to that of her index patient with respect both to the number of testing sessions and the inter-test intervals. The number of treatment cycles varied from 4 to 8 and the number of post-baseline assessment sessions ranged from 4 to 6 (AC-T was a dose-dense regimen involving 8 treatments 2 weeks apart and assessments were conducted after every 2 cycles). All assessments were administered by one of two examiners with extensive training in neuropsychological testing. Any given participant was

seen by the same examiner at every study visit, usually in the participant's home. Psychometric instruments were administered in a set order. Medical records were reviewed periodically throughout the study. Approximately one-third of the sample participated in a companion functional magnetic resonance imaging study, the results of which will be published separately. Women were paid \$30.00 for each study visit and were provided with individual feedback on their cognitive test results at the end of the study.

Neuropsychological Measures

Standard Neuropsychological Tests: The pencil-and-paper neuropsychological test battery was about 60 minutes in duration. The tests were selected to focus on the areas of cognition previously shown to be sensitive to the effects of cancer treatments (Reddy, L., 2005) and to correspond to the cognitive domains covered by the computerized test battery. The selected neuropsychological tests all have established reliability, validity and sensitivity to subtle cognitive impairment (Lezak, M., et al., 2004; Strauss, E., 2006) and conform to recent recommendations of the International Cognition and Cancer Task Force (Wefel, J. et al, 2011).

Computerized Cognitive Tests: The computerized cognitive test, CNS-Vital Signs (CNS-VS) [59-60], was administered upon completion of the pencil-and-paper neuropsychological measures. We selected a battery of CNS-VS subtests measuring attention, reaction time, working memory, executive function, and visual and verbal episodic memory that took approximately 30 minutes to administer. CNS-VS is user friendly, has been validated in a cancer population, and has been proven sensitive in monitoring an individual's cognitive status over time. It has numerous parallel forms with established reliability for all of the core tasks. We administered the PC version of the test on an IBM laptop computer.

Psychosocial Measures: Psychological distress was measured using the Beck Depression Inventory-II (BDI-II) (Beck, A., et al, 1996) and the Profile of Mood States (POMS) (Bremner, J., et al., 1999). The BDI-II is a self-report questionnaire with demonstrated reliability and validity in measuring severity of depression in adolescents and adults (Lezak, M., et al., 2004; Strauss, E., 2006; Beck, A., 1996). It is comprised of 21 multiple-choice items corresponding to prevailing diagnostic criteria for depression. Total score, reflecting the sum of the item ratings, was used for current analyses. The POMS consists of 65 adjectives corresponding to 6 dimensions reliably identified by factor analytic studies: Tension-Anxiety, Depression-Dejection; Anger-Hostility, Vigor-Activity, Fatigue-Inertia, and Confusion-Bewilderment. Respondents rate each adjective on a 5-point scale with reference to their mood state over the previous week. For the current analyses, we used a Total Mood Disturbance Score (POMS TMD) which reflects the scores on all dimensions. Note that POMS TMD reflects both mood state and fatigue. The POMS has also been shown to have satisfactory reliability and validity (Lezak, M., 2004; Bremner, J., et al., 1999).

Appendix C

INFORMATION SHEET AND CONSENT FORM

A prospective dose-response and fMRI study of the effects of chemotherapy on neural function in early-stage breast cancer patients**Purpose**

Many cancer patients who have received chemotherapy experience some changes in their mental functioning. They refer to this as “chemo fog” or “chemo brain”. This study is being done to further examine how chemotherapy affects brain function and mental processes by comparing brain function in women with breast cancer who are undergoing chemotherapy to women without breast cancer.

Your participation in this study would last approximately 18 months, and would involve repeated testing of *cognitive* functions (i.e., mental faculties such as attention and memory), completion of various rating scales concerning your physical and emotional state, and sampling of saliva for hormone measurement. Some participants will also have 3 functional magnetic resonance imaging (fMRI) brain scans. These procedures are described in greater detail below.

Participants

This study will compare a group of women with early stage breast cancer who are undergoing chemotherapy to a group of women without breast cancer. In an effort to have these groups be as similar as possible, we will ask participants with breast cancer if they have a friend or family member similar in age and education who might also like to participate in the study, and serve as their control. We will try to arrange for you to come to your study appointments together, if you would like.

Procedures***Cognitive Testing***

Participants in this study will undergo a series of computerized and pencil-and-paper tests to measure cognitive functions. This testing will be done prior to beginning chemotherapy, following each chemotherapy session, and again one year after completion of chemotherapy (or at equivalent time points in women without breast cancer). It is estimated that each session will take 75-90 minutes, except for the first session, which will take approximately 2-1/2 hours. This testing can be done at the Ottawa Hospital (Civic or General Campus) or at your home, whichever you prefer.

Questionnaires

At the time of the cognitive testing, you will also be asked to complete some questionnaires concerning your physical and emotional well-being (included in the 75-90 minutes).

Valid until July 28, 2011

Version 3 (01 Dec 08)

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fMRI Scans

You will also be asked to undergo repeated fMRI scanning. You do not have to participate in the fMRI component of the study in order to take part in the cognitive testing component; however, participation in the fMRI component is contingent upon your involvement in the cognitive testing portion of the study. If you do agree to the fMRI component, you will undergo scans on 3 occasions: prior to beginning chemotherapy, following the last chemotherapy cycle, and one year after completion of chemotherapy (or at equivalent time intervals for those women not undergoing cancer treatment). fMRI is a painless scanning procedure that allows visualization of your brain using magnetic field and radiowaves. It shows the activity in different areas of the brain by measuring blood flow to those areas. Areas with greater blood flow are more active. By scanning the brain while an individual is performing various mental tasks, we can get information about the parts of the brain that are involved in those tasks. Therefore, you will be asked to perform various mental activities while you are in the scanner.

You will be required to go to St-Joseph MRI (228 St-Joseph Blvd., Suite 203, Gatineau) for the fMRI scans. Transportation will be provided if you wish, and a member of the study team will be present for your visit. Each visit will take about one hour, and you will be in the scanner for about 35 minutes.

During the scan, you will be asked to lie flat with your head stabilized on an automated bed that is moved into a two-foot wide cylinder (the scanner). While you are lying in the scanner, you will be asked to do a variety of mental tasks that will be presented on a screen located at your feet. There will be a mirror on the scanner to ensure that you can clearly see the screen. You will be asked to respond to the items on the computer screen by pressing a button with your finger. The types of tasks done in the scanner will be similar to the computerized mental tests done in the cognitive component of the study.

A neuroradiologist will review all of the fMRI scans. Should any abnormality be detected on your scan, you will be notified immediately. If you have breast cancer, your oncologist will also be informed.

Hormone Testing

It has been suggested that hormones, such as estrogen and cortisol, may have a role in the effect of chemotherapy on mental function. Therefore, at each cognitive testing session, you will be asked to provide a small amount of saliva in a vial in order to measure estrogen levels. If you choose to participate in the fMRI portion of the study, you will also have cortisol measured from your saliva. For the cortisol testing, you will take samples of your own saliva at home (by

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moistening a cotton swab in your mouth) at various times over a two day period. You will be provided with materials and instructions for this procedure when you go for your fMRI.

Possible Risks

There are no particular risks associated with psychological testing. Some people do experience performance anxiety in testing situations. Every effort will be made to alleviate this.

The fMRI procedure is also low risk for most people. Nothing is injected into you, no incisions will be made, and you will **NOT** be exposed to any radiation. Individuals with metal implants (such as a pacemaker, aneurysm clip, or metal dental implants beyond fillings) cannot take part in this study. The technician will review this carefully with you prior to scanning.

The area within the scanner is quite confined, and this may cause discomfort for some people. If you feel uncomfortable during the fMRI, you can talk to the technician who will be in continuous two-way communication with you. You will also have a button with you at all times that you can press if you wish to exit the scanner. The scanner makes a loud humming sound with occasional louder noises. You will be given earplugs and headphones to wear in the scanner to help reduce this noise.

Possible Benefits

Costs/Remuneration/Compensation

As a participant in this study you will have an opportunity to learn more about your intellectual, emotional, and neural functioning. If you wish, you will be given feedback about this at the end of the study. If you participated in the fMRI portion of the study, you may also have copies of your brain scans if you wish. The only other benefit to participants is the knowledge that they have contributed to our understanding of possible side effects of chemotherapy and, perhaps, to the quality of care available to future patients.

You will be paid \$30 for each study visit, in order to cover any expenses that you may incur. You will be provided with a day parking pass for each hospital visit related to the study.

Income earned as a result of your participation in this study that is not for reimbursement of study expenses will be considered taxable income by Revenue Canada. In order to receive payment for your participation in this study, it will be necessary to provide the investigator or their delegate with your Social Insurance Number. The Ottawa Hospital will then issue a T4A for any amount over \$500.00 by the end of February of the following year.

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In the event of research related side effects or injury, you will be provided with appropriate medical treatment. By participating in the study, you are not waiving any legal rights that may be available to you. The study doctor and the hospital still have their legal and professional responsibilities.

Confidentiality

Personal health information obtained from this study will be kept confidential, unless release is required by law. The data from this study will be stored in a computerized database. Your data will be identified by an independent study number only. Your name will not appear on any study document or computer file. Apart from the investigators of this study, only the Ottawa Hospital Research Ethics Board and the Ottawa Health Research Institute may review your relevant study data and medical records (in order to check that the study is being performed properly and that you have given your full informed consent). The collective results of the study may be presented or published, but the data will be anonymous. No identifiable information will leave The Ottawa Hospital without your consent.

Right to Withdraw

Your participation in this study is entirely voluntary. You may choose to participate in the cognitive testing component of the study without participating in the fMRI component (but not *vice versa*). Even if you consent to begin this study, you are free to withdraw at any time. Your decision not to participate or to withdraw will not affect your care at the Ottawa Hospital, now or in the future.

Questions

If you have any questions or require further information about this study, do not hesitate to contact the investigators, Dr. Barbara Collins, or Dr. Andra Smith, or the study coordinator, Ms. Joyce MacKenzie.

This study has been approved by the Ottawa Hospital Research Ethics Board. The Board considers the ethical aspects of all research projects involving human subjects that are conducted at the Ottawa Hospital. If you have any questions about your rights as a research subject, you may contact the Chairman of this Board.

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Signatures

I have read this 4-page consent form, and the study and related procedures have been explained to me in terms I can understand. I have had a chance to ask any questions that I had and my questions have been answered to my satisfaction. I have had enough time to think about the information discussed with me.

My signature below indicates that I agree to participate in this study until such time as I decide otherwise. I will receive a signed copy of this informed consent to keep as a reference about the study.

Participant (Print Name)

Signature

Date of Signature

Investigator Delegate
(Print Name)

Signature

Date of Signature

Appendix D

Response pad in scanner

