A Prospective Neuroimaging Study of Chemotherapy-Related Cognitive Impairment in Breast Cancer Patients

Christian Lepage

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Psychology
Social Sciences
University of Ottawa

Abstract

Complaints of reduced cognitive abilities are frequent following chemotherapy. Research in the breast cancer population has revealed some patients may experience treatment-related decline in cognitive domains such as executive function, information processing speed, memory and learning, attention and concentration, and working memory. The extent and mechanism of action of this phenomenon remain poorly understood. Neuroimaging research can characterize the neural underpinnings of chemotherapy-related cognitive impairment; however, with few longitudinal studies, more prospective studies are needed to elucidate this important topic. The aim of this thesis was to use magnetic resonance imaging and contemporary analysis techniques to better understand the influence chemotherapy exerts on both the brain and cognition. This was achieved in two studies that measured cognitive function and brain structure and function at three time points: pre-treatment, one month post-chemotherapy, and at one-year follow-up. In the first study, the association between regions of brain structural changes and cognitive function was examined. The second study took a narrower approach and investigated the functional profile of brain activity during a working memory task. Patients had more pronounced structural and functional disruptions shortly after treatment, relative to both pre-treatment and one-year post-chemotherapy intervals. Regions of structural compromise were largely associated with information processing speed. Functional disruptions occurred in a frontoparietal network. Overall, this thesis provides more evidence of the injurious role chemotherapy plays on cognition, particularly in the short term. This thesis also provides the first longitudinal neuroimaging study to illustrate a complete resolution of working memory related brain disruption one year post-treatment.

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Legend

ACT	Auditory Consonant Trigrams
APOE E	Apolipoprotein E
ANOVA	Analysis of Variance
BA	Brodmann Area
BBB	Blood Brain Barrier
BOLD	Blood-Oxygen Level Dependent
COMT	Catechol-o-methyltransferase
COWAT	Controlled Oral Word Association Test
CRCI	Chemotherapy-related Cognitive Impairment
CNS-VS	CNS-Vital Signs
dlPFC	Dorsolateral Prefrontal Cortex
DNA	Deoxyribonucleic Acid
FEC	fluorouracil, epirubicin, and cyclophosphamide
FDR	False Discovery Rate
fMRI	Functional Magnetic Resonance Imaging
FPN	Frontoparietal Network
GLM	General Linear Model
HLM	Hierarchical Linear Modeling
MNI	Montreal Neurologic Institute
MRI	Magnetic resonance imaging
PASAT	Paced Serial Addition Test
RNA	Ribonucleic Acid
ROI	Region of Interest
SLF	Superior Longitudinal Fasciculus
SPM	Statistical Parametric Mapping
TE	Echo Time
TNF	Tumour Necrosis Factor
TR	Repetition Time
VBM	Voxel-based Morphometry
WAIS	Wechsler Adult Intelligence Scale
WRAT-3	Wide Range Achievement Test

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Ethical Standards

A copy of the original approval letter from the Ottawa Hospital Research Ethics Board is included as Appendix A.

Statement of Co-Authorship

The included manuscripts were crafted with the guidance of Dr. Andra Smith, my thesis supervisor. As the primary author, I was responsible for the formulation of the research questions, selection of methods and statistical analyses, data collection, and preparation of the manuscripts. Dr. Smith provided guidance and assistance in all aspects of this project. Carole Scherling and Barbara Collins aided in the conceptualization of the overarching research project from which these studies were drawn, and they provided valuable feedback on the manuscripts and analyses. Barbara Collins and Joyce MacKenzie oversaw the neuropsychological data collection. Jeremy Moreau provided assistance with data analysis of the first study. Nancy Wallis, Emily Barlow-Krelina, Carole Scherling, Miranda Kiyomi Setoguchi, and Zahra Mawani aided data collection.

Introduction

Breast cancer is the most frequently diagnosed cancer among women, afflicting an estimated 1.67 million women worldwide (Ferlay et al., 2013) and nearly doubling the next-most common female cancer (Jemal et al., 2011). This trend is mirrored in North America where the estimated incidence of new cases of breast cancer in 2014 was 232,670 in the United States (Siegel, Ma, Zou, & Jemal, 2014) and 24,400 in Canada (Canadian Cancer Society, 2014). Breast cancer survivorship rates have increased over the decades, due in part to the development of improved treatment options. Since 1986, the five-year relative survival rate for Canadian breast cancer patients increased from 76% to 88% (Canadian Cancer Society, 2014). Chemotherapy-based adjuvant therapies supplement principal surgery-based interventions and have become commonly employed, resulting in greater survivorship among breast cancer patients (Mariotto et al., 2002).

Subsequent to adjuvant chemotherapy exposure, many breast cancer patients report cognitive decline (e.g. see (Pullens, De Vries, & Roukema, 2010)), a phenomenon colloquially referred to as “chemofog” and “chemobrain” (Raffa et al., 2006). Self-perceived deterioration in mental functioning can adversely impact work and family life for breast cancer survivors (Boykoff, Moieni, & Subramanian, 2009). Objective evidence of chemotherapy-related cognitive impairment (CRCI) in breast cancer patients has mounted in the last decades. Varying degrees of cognitive under-performance are found in many domains, including working memory, information processing speed, visuospatial ability, attention and concentration, motor functioning, executive function, and memory (Ahles et al., 2002; Bender et al., 2006; Brezden, Phillips, Abdolell, Bunston, & Tannock, 2000; Falleti, Sanfilippo, Maruff, Weih, & Phillips, 2005; Fan et al., 2005; Hurria, Rosen, et al., 2006; Jansen, Cooper, Dodd, & Miaskowski, 2011;

Kam et al., 2015; Reid-Arndt, Hsieh, & Perry, 2010; van Dam et al., 1998; Wefel, Saleeba, Buzdar, & Meyers, 2010).

The etiology of CRCI is complex. CRCI is subtle, appears to affect subgroups of patients only, and is a transient phenomenon for some (Falleti et al., 2005; Stewart, Bielajew, Collins, Parkinson, & Tomiak, 2006). Despite having the greatest influence on cognitive functioning during and up to 6 months post treatment (Collins, Mackenzie, Tasca, Scherling, & Smith, 2014; Jim et al., 2012), chemotherapy is related to impaired cognition decades after treatment (Ahles et al., 2002; Koppelmans et al., 2012). Furthermore, cognitive dysfunction has been found prior to the commencement of chemotherapy (Ahles et al., 2008; Jansen et al., 2011; Wefel, Lenzi, Theriault, Davis, & Meyers, 2004), hinting at other contributing factors to CRCI, including the cancer itself and emphasizing the need for increased study using other investigative methods.

The neurophysiological correlates of CRCI have been a topic of increased study in recent years. Prospective neuroimaging studies of brain structure have found abnormalities in both grey and white matter compartments following chemotherapy (Deprez et al., 2012; McDonald, Conroy, Ahles, West, & Saykin, 2010; McDonald, Conroy, Smith, West, & Saykin, 2012). Although some recovery has been noted one year from treatment (McDonald et al., 2010), retrospective studies have reported brain matter disruption decades after chemotherapy (de Ruiter et al., 2012; Koppelmans et al., 2012, 2014). Additionally, there is conflicting evidence of pre-treatment brain structure anomalies, with some support for (McDonald, Conroy, Smith, et al., 2012) and against (McDonald et al., 2010) baseline differences between breast cancer patients and control participants before patient adjuvant treatment.

Similarly, functional brain differences have been reported prior to chemotherapy (Askren et al., 2014; Berman et al., 2014; Cimprich et al., 2010; Scherling, Collins, Mackenzie, Bielajew,

& Smith, 2011, 2012). Analogous to the neuropsychological and neuroanatomical literature of CRCI, evidence suggests that the greatest neurofunctional abnormality is apparent during and shortly after chemotherapy, relative to baseline and one-year after treatment (McDonald, Conroy, Ahles, West, & Saykin, 2012). Notably, retrospective studies have reported differences in neural recruitment during cognitive tasks between breast cancer patients and healthy controls over 10 years after chemotherapy (de Ruiter et al., 2011; Stouten-Kemperman et al., 2015). These mixed findings further suggest the reduced cognition experienced by breast cancer patients during the course of chemotherapy may be influenced by disease and other treatment related factors.

In light of the work showing brain structure and function irregularities in breast cancer patients, there is an impetus to examine the brain networks that support cognitive functioning. The few studies that have examined functional networks in breast cancer patients reflect the findings from other neuroimaging modalities, with breast cancer patients showing decreased overall network efficiency compared to control participants (Bruno, Hosseini, & Kesler, 2012; Hosseini, Koovakkattu, & Kesler, 2012; Kesler et al., 2013). To date, only one pilot study has longitudinally examined network integrity in breast cancer patients (Dumas et al., 2013). The results of that study suggest the default mode and dorsal attention networks are disrupted after chemotherapy, although the former has displayed some recovery one year after chemotherapy. Nevertheless, there is a lacuna in the CRCI neuroimaging literature of prospective brain network integrity investigations.

Thus, the overall aim of this thesis was to use contemporary neuroimaging techniques to elaborate the current scientific understanding of the neural mechanisms that underlie CRCI. In order to achieve this goal, this thesis was divided into two studies, each having their own set of objectives germane to elucidating the influence that chemotherapy exerts on cognition and brain

function and structure. The first study used a neuroimaging analysis technique to explore the course of grey matter volume changes in breast cancer patients and investigate the relationship between regions of grey matter loss and cognitive function. In the second paper, the integrity of a working memory network is examined prospectively - the first such study in the CRCI literature. The two studies are presented in manuscript format, with the structural investigation appearing as it does in published format, and the functional study appearing as it did during submission to a peer-reviewed journal. A general discussion follows the articles, summarizes the findings of the thesis, reviews the implications, acknowledges the limitations of the work, and offers direction for future research. Before presenting the articles, a general introduction provides the necessary background information.

Chemotherapy

Chemotherapy drugs used to treat breast cancer can be classified into different groupings, although some of them have characteristics that overlap two or more categories. Various types of chemotherapy agents may be used, depending on the stage of cancer and treatment response (DiPiro, 2009); however, only the most commonly used drugs for early stage breast cancer will be discussed. Alkylating drugs disrupt the replication and copying processes during the cell cycle, precluding cancerous cells from repairing damaged DNA (Gerson, Bulgar, Weeks, & Chabner, 2011). A commonly used alkylating agent in breast cancer is cyclophosphamide (Morris & Hudis, 2011). Platinum-based antineoplastic drugs are sometimes grouped with alkylating agents since they destroy cells in a similar fashion. Frequently used platinum-based drugs in the treatment of breast cancer include cisplatin and carboplatin (Decatris, Sundar, & O'Byrne, 2004). Antimetabolites supplant the normal building blocks of RNA and DNA during the chromosomal copying phase of the cell cycle, interfering with cell growth. The

antimetabolites used in breast cancer treatment are 5-fluorouracil and methotrexate (Hortobagyi, 2000). Anthracyclines subvert mitosis throughout the cell cycle by disrupting enzymes involved in DNA replication (Skeel, 2011). Doxorubicin and epirubicin are regularly used anthracyclines in breast cancer treatment. Finally, the taxanes - docetaxel and paclitaxel - are plant alkaloids that interfere with microtubules, leading to mitotic arrest and, thus, inhibited cell division (Skeel, 2011). Contemporary regimens employ combinations of chemotherapy drugs to optimize outcomes for breast cancer patients.

Chemotherapy may be administered preoperatively and after surgical intervention for breast cancer. It may be delivered in concert with a combination of other adjuvant interventions, including radiation and hormonal therapies (National Cancer Institute, n.d.). Prior to surgical removal of the cancerous tissue, neoadjuvant chemotherapy may be dispensed to shrink a tumour that is currently inoperable or to allow for breast-conserving surgery (Mauri, Pavlidis, & Ioannidis, 2005). Adjuvant therapies, including chemotherapy, are delivered to destroy cancer cells or to stop them from dividing (Office on Women's Health, n.d.). Chemotherapy can be administered orally and via injection, whether it is given prior to or following surgery. Treatment occurs for three to six months in weeks-long cycles that vary depending on the drugs used (American Cancer Society, n.d.).

Postulated mechanisms of impairment

Neurotoxic sequelae have been reported for nearly each category of the chemotherapy agents (Dropcho, 2004; Scatchard & Lee, 2010; Schagen, Muller, Boogerd, Mellenbergh, & van Dam, 2006); however, the mechanisms of chemotherapy neurotoxicity remain to be well characterized. Several processes have been proposed, including direct toxic cellular effects,

indirect metabolic abnormalities, inflammatory processes, and vascular influences (Dietrich, 2010).

Blood-brain barrier. The blood-brain barrier (BBB) is generally thought to provide protection from cytotoxic agents. In fact, it is a major obstacle for the delivery of chemotherapy agents to brain-based tumours and micrometastatic disease (Deeken & Löscher, 2007). Despite this, there is some evidence that 5-fluorouracil has a degree of BBB permeability in rodents (Sakane, Yamashita, Yata, & Sezaki, 1999). The BBB may be susceptible to increased permeability via the effects of cancer (e.g. micrometastases), radiation treatment, or genetic variability (Ahles & Saykin, 2007; Wefel, Witgert, & Meyers, 2008). It has been speculated that a synergistic effect of repetitive exposure to combination of chemotherapy drugs may also impact the blood-brain barrier in a way that allows lipophilic drugs to penetrate the brain parenchyma; however, this hypothesis remains to be supported empirically, particularly in humans (Dietrich, 2010).

Oxidative stress. Oxidative stress is a biochemical condition in which there is an imbalance between cellular pro-oxidants and antioxidants that leads to irregular cellular signalling. It has been related to cancer, inflammation, aging, drug action, and drug toxicity (Sies, 1985). Damage to DNA in neuronal cells from oxidative stress is common (Park et al., 1998) and may occur via exposure to exogenous toxins (Ozben, 2007). For example, the anthracycline agent doxorubicin - commonly used to treat breast cancer - is associated with the induction of oxidative stress (Quiles, Huertas, Battino, Mataix, & Ramírez-Tortosa, 2002). A recent study by Conroy et al. (2012) found increased oxidative DNA damage in early-stage breast cancer patients compared to healthy control participants at an average of six years post-chemotherapy. Notably, the majority (71%) of the regimens included in that study incorporated

doxorubicin. Furthermore, the authors found that oxidative DNA damage in the patient group was associated with reduced grey matter density in temporal and mesencephalic regions. Similarly, reduced hippocampal volume has been related to increased levels of tumour necrosis factor (TNF)-alpha in early-stage breast cancer patients (Kesler, Janelsins, Koovakkattu, & Palesh, 2013). It has been proposed that TNF-alpha, which can pass the BBB and is increased by doxorubicin, can induce the expression of nitric oxide synthases. In turn, nitric oxide synthases can then lead to oxidative stress (Chen, Jungsuwadee, Vore, Butterfield, & St Clair, 2007). Nevertheless, it is not well understood how DNA damage leads to injury in the brain (Ahles & Saykin, 2007). Oxidative DNA damage has been linked with neurodegenerative disorders that are characterized by cognitive symptoms (Keller et al., 2005; Wang et al., 2014), suggesting a relationship between DNA damage and cognitive difficulties.

Proinflammatory cytokine dysregulation. Cytokine is a general term used to describe small proteins that are extruded from cells and have a distinct impact on intercellular interaction and communication. Cytokines may interact with host, neighbouring, or remote cells. At times, they are referred to with function-specific terms: a chemokine is a cytokine with chemotactic activities, and interleukin is a cytokine made by one leukocyte that acts on other leukocytes. A cytokine may stimulate the production of another in a remote cell, resulting in cascaded manufacturing. As part of the immune system, many cell types produce cytokines, including B cells, T cells, macrophages, mast cells, neutrophils, basophils, and eosinophils (Zhang & An, 2007).

Increased levels of cytokines have been reported in cancer patients treated with standard-dose chemotherapy (Janelsins et al., 2012; Lotti et al., 2013). Cytokine-induced inflammation related to chemotherapy has been linked to cognitive diminishment (Janelsins et al., 2012) and

self-reported memory complaints (Ganz et al., 2013). As mentioned, increased cytokine levels (e.g. TNF-alpha) have been related to lower hippocampal volume in breast cancer patients (Kesler et al., 2013). The authors of that study also reported reduced levels of another cytokine, interleukin-6, was linked to smaller hippocampal volume, with both cytokines being associated with memory performance. Using positron-emission tomography, a recent pilot study examined the relationship between frontal lobe metabolism and inflammatory markers, including C reactive protein and interleukin-6 (Pomykala et al., 2013). The authors found that at baseline (which included measures taken post-chemotherapy), the presence of inflammatory markers was related to left inferior frontal and right inferior lateral metabolic activity; there was no such relationship for control participants. At one-year follow-up, frontal and medial metabolic activity remained positively correlated with inflammatory markers in patients; again, there was no link between these markers and neurometabolic activity in control participants. It has been suggested that cytokines may be triggered by DNA damage (e.g. caused by chemotherapy drugs), which could establish a cycle of further DNA damage and cytokine activity, resulting in chronic inflammation. The inflammation can increase oxidative stress, further compounding this cycle (Ahles & Saykin, 2007). Overall, the evidence suggests that cytokine deregulation may be related to CRCI; however, more evidence must accrue before such a relationship can be deemed definitive.

Risk Factors

CRCI affects a subset of breast cancer patients treated with chemotherapy, suggesting that the cognitive effects experienced after treatment may be modulated by one or more risk factors. Some risk factors are supported by evidence of a deleterious influence on cognition independent of chemotherapy. These may predispose some patients to CRCI. Other risk factors

arise from disease and treatment processes that are variable across breast cancer patients. Potential influences of CRCI that may emerge outside of the processes related to cancer and its treatment will be discussed first.

Age. Age-related changes in cognition are well-accepted phenomena in the neuropsychological literature (Salthouse, 2009). The risk of being diagnosed with breast cancer rises with age, with the highest rates occurring at age 60 and later (Key, Verkasalo, & Banks, 2001). Unsurprisingly, studies of CRCI predominantly include samples whose mean age is above 50 years, necessitating a need to control for age-related changes in cognition. Thus far, it has been common practice in the CRCI literature to control for age (e.g. see (Scherling & Smith, 2013)). Yet, it has been speculated that chemotherapy may hasten aging and that it can induce a vulnerability to late-emerging cognitive decline (Schagen & Wefel, 2013). Correspondingly, cross-sectional studies conducted many years after patient chemotherapy have reported CRCI in breast cancer patients relative to both healthy and chemotherapy-untreated controls (Ahles et al., 2002; Koppelmans et al., 2012; Yamada, Denburg, Beglinger, & Schultz, 2010). Although impairment is reported after age has been controlled for, the cross-sectional nature of these studies does not preclude the possibility that chemotherapy administration may place women at greater risk for cognitive decline in the future by hastening age-related changes in cognition.

Age is positively related to executive dysfunction in chemotherapy-treated patients (Kesler, Kent, & O'Hara, 2011) and with a high degree of memory complaints (Hurria et al., 2006). Similarly, older age is associated with increased toxicity from chemotherapy exposure (Hurria et al., 2011; Muss et al., 2007). Thus, it may be that the effects of chemotherapy vary depending on the age of the patient at the time of administration, such that older women may be at greater risk of treatment-related cognitive decline than younger ones.

Cognitive reserve. In response to increased cognitive demands, there is variability in the magnitude and efficiency of the associated neural response across individuals. One's ability to optimize performance via flexible recruitment of brain networks, proposed to reflect the use of alternative cognitive strategies, has been labelled *cognitive reserve* (Stern, 2002). Cognitive reserve has been postulated to account for the differential outcome among individuals following brain injury, such that those with a higher degree of it have less functional impairment than individuals with lower levels (Stern, 2009). Using WRAT-3 (Wilkinson, 1993) reading scores as a measure of baseline cognitive reserve, Ahles et al. (2010) showed that chemotherapy-treated breast cancer patients - who were older and had lower pre-treatment cognitive reserve - had reduced information processing speed compared to control participants. Similarly, pre-treatment executive network inefficiency is related to breast cancer patients' complaints of disrupted cognition and elevated fatigue (Askren et al., 2014).

Educational level is related to cognitive reserve, with higher education acting as a protective factor against neurodegenerative disorders in old age (Carret et al., 2003). Higher educational attainment in chemotherapy-treated breast cancer patients is linked to decreased perseverative errors during executive function tasks (Kesler et al., 2011). Although studies of cognitive reserve are scarce in the CRCI literature, taken together, current evidence suggests that it modulates the influence of chemotherapy on cognition.

Genetic factors. Increased susceptibility to brain insult following chemotherapy administration may be, to an extent, facilitated by genetic variability in genes that manage neural repair (e.g. see (Ahles & Saykin, 2007)). Apolipoprotein E (APOE) is a component of various lipoproteins, and its primary role is to transport lipids and cholesterols throughout the body. It is the major apolipoprotein expressed in the brain, where it has the added functions of mediating

synaptogenesis, synaptic plasticity, and neuroinflammation (Chouraki & Seshadri, 2014). The APOE E4 allele has been identified as a risk factor for the development of Alzheimer's disease and cognitive decline in carriers not diagnosed with mild cognitive impairment or Alzheimer's disease (Liu, Liu, Kanekiyo, Xu, & Bu, 2013). Although scarce, there is evidence that the APOE E4 allele in chemotherapy-treated breast cancer patients is related to decreased cognition compared to non-carriers (Ahles et al., 2003, 2014). However, evidence of neuroimaging markers of CRCI where APOE E4 status was explored remains equivocal (Ferguson, McDonald, Saykin, & Ahles, 2007; McDonald, Conroy, Smith, et al., 2012).

Catechol-o-methyltransferase (COMT) is an enzyme that regulates catecholamine neurotransmitters, such as dopamine, epinephrine, and norepinephrine through deactivation. COMT enzymes are coded by the COMT gene, which has several alleles, including Val158Met. This single-nucleotide polymorphism substitutes valine (e.g. Val) with methionine (e.g. Met) at codon 158 (Lachman et al., 1996). A Val allele of COMT can increase the catabolism of dopamine by a factor of four, in contrast to that in COMT-Met homozygote carriers. The Val158Met allele has been linked with dopamine levels in the prefrontal cortex, and a reduced level of the neurotransmitter in that area may account for the reports of disparate cognition between COMT-Val and -Met carriers, specifically on tasks of attention and executive function (Dickinson & Elvevåg, 2009). A retrospective study of CRCI found that COMT-Val carriers in a breast cancer group with a history of chemotherapy exposure performed more poorly on tests of overall cognition, complex cognition, attention, verbal fluency, and motor speed, relative to their COMT-Met carrying counterparts (Small et al., 2011). Although the differences were not statistically significant after correcting for multiple comparisons, the study suggests that breast cancer patients who are carriers of COMT-Val may be especially vulnerable cognitively to

CRCI. Overall, the study of genetics in CRCI is nascent, and more studies should be conducted to convincingly determine what role, if any, genes might play in rendering some breast cancer patients more susceptible than others to cognitive decline following chemotherapy.

Dosage. There is evidence that CRCI may be modulated by the magnitude and duration of treatment, as well as the number of chemotherapy cycles. An early study of CRCI compared the effects of high-dose to standard-dose chemotherapy on cognition in breast cancer patients (van Dam et al., 1998), and found nearly twice the rate of impairment in the former treatment arm. Additionally, the women who received high-dose chemotherapy were 8.2 times more likely than chemotherapy-free controls to experience cognitive impairment. A later prospective study supported those findings, demonstrating that breast cancer patients receiving high-dose chemotherapy experienced significant cognitive deterioration from pre-treatment baseline to six months afterwards, relative to healthy controls (Schagen et al., 2006). In contrast, breast cancer patients who had received either a standard-dose treatment or were chemotherapy-free did not have a significant decline of cognition relative to healthy controls. Evidence of dose-modulated CRCI prompted the investigation of cumulative effects of chemotherapy on cognition. In a novel approach to studying CRCI, Collins et al. (2013) measured the cognitive functioning of breast cancer patients and controls at pre-treatment baseline, and following patients' individual chemotherapy cycles. The authors found that, although the patient group did not significantly decline on most neuropsychological measures, it did not benefit from repeated testing to the extent that the healthy control group did. In fact, once practice effects were controlled for, scores on a composite of overall cognition diminished with successive chemotherapy administrations. Interestingly, this pattern was observed in a subset of the authors' larger sample, which consisted of a group treated with 5-fluorouracil, epirubicin, and cyclophosphamide (FEC) - the regimen

that was designated as standard-dose in the aforementioned studies in this section (e.g. (Schagen et al., 2006; van Dam et al., 1998)). Thus, although women who receive higher doses of chemotherapy are at increased risk of CRCI, successive administrations of chemotherapy, even in low-dose regimens, can also render breast cancer patients susceptible to disrupted cognitive function.

Other adjuvant therapies. The treatment of breast cancer is often multifaceted, with chemotherapy being one of a number of adjuvant interventions employed concurrently or in succession to remove the disease. Radiotherapy may be indicated in early stage breast cancer subsequent to lumpectomy or mastectomy, with an aim to eliminate any cancerous cells that have lingered or spread to other areas (American Cancer Society, 2015). The cognitively injurious effects of radiotherapy are well recognized across many cancer populations, including breast cancer (Jim et al., 2009; Quesnel, Savard, & Ivers, 2009; Shibayama et al., 2014; Small et al., 2011), with neurological and neuropsychological compromise frequently being the dose-limiting consideration of this treatment. In fact, radiotherapy is generally not administered concurrently with chemotherapy due to potential synergistic effects that can exacerbate the negative consequences associated with these interventions (Bellon & Harris, 2005).

Hormonal therapy is generally indicated for estrogen-positive tumours, due to its ability to treat hormone-sensitive breast cancer by blocking ovarian function, estrogen production, or the effects of estrogen (National Cancer Institute, 2012). With most breast cancers being estrogen-receptor positive (Anderson, Chatterjee, Ershler, & Brawley, 2002), hormone therapy is commonly administered to breast cancer patients. There are inconsistent findings in the CRCI literature regarding the cognitive effects of hormonal therapy. Some prospective studies report that there are no cognitive differences between patients treated with chemotherapy and hormone

therapy (Fan et al., 2005; Jenkins et al., 2006), while others have found that the latter was associated with greater, widespread compromise (Bender et al., 2006; Collins, Mackenzie, Stewart, Bielajew, & Verma, 2009). Neuroimaging evidence has supported findings in favor of cognitive disruption following hormone therapy, with one study showing that breast cancer patients who had taken tamoxifen had smaller hippocampal volumes and diffuse hypometabolism in frontal brain areas (Eberling, Wu, Tong-Turnbeaugh, & Jagust, 2004). Thus, within the context of the CRCI literature, disentangling chemotherapy's contribution to post-treatment cognitive dysfunction can be confounded by the presence of chemotherapy along with a combination of radiotherapy and hormone therapy (e.g. see (Tager et al., 2010)).

As evidenced by the array of potential mechanisms of impairment and risk factors, all of which can vary from one patient to the next, CRCI is a complex phenomenon requiring further clarification.

Cognition and chemotherapy

Although earlier, cross-sectional studies first raised awareness about CRCI (van Dam et al., 1998; Wieneke & Dienst, 1995), longitudinal investigations, incorporating a pre-treatment baseline, importantly showed that breast cancer patients could have clinically significant reductions in cognitive function over time, despite an absence of cross-sectional between-group differences, relative to controls (e.g. see (Schagen et al., 2006)). A large number of longitudinal studies of CRCI have now been conducted, comparing cognitive performance in chemotherapy-treated patients to both chemotherapy-free and healthy controls (Ahles et al., 2010; Bender et al., 2006; Collins et al., 2009; Fan et al., 2005; Hermelink et al., 2007; Jenkins et al., 2006; Quesnel et al., 2009; Tager et al., 2010). Among longitudinal, prospective investigations, CRCI in patients receiving adjuvant chemotherapy has been found in 12 to 82% of samples, with the

cognitive domains of executive function, working memory, information processing speed, visuospatial ability, attention and concentration, motor functioning, and memory frequently impacted. The wide range of incidence has been speculated to reflect small sample sizes, heterogeneous use of neuropsychological assessment batteries and control groups, the presence of various treatment regimens, and disparate cut-offs used to demarcate impaired from normal cognitive functioning (Janelsins, Kesler, Ahles, & Morrow, 2014). In spite of this variability, it has become evident that cognition is adversely impacted in some breast cancer patients following chemotherapy.

Of the commonly reported cognitive domains affected by chemotherapy, working memory appears to be the most vulnerable (Bender et al., 2006; Collins et al., 2009; Shilling, Jenkins, Morris, Deutsch, & Bloomfield, 2005; Stewart et al., 2006). Across a broad range of cognitive domains, working memory was uniquely lower among chemotherapy-treated patients compared with hormone-therapy-only controls (Stewart et al., 2008). Interestingly, both groups scored within normal limits on testing; however, only the chemotherapy-treated group experienced reliable cognitive deterioration. Although the mechanisms of impairment in CRCI remain to be understood, animal models suggest that chemotherapy-driven neuroinflammation may produce a neurotoxic effect on white matter integrity via demyelination (Briones & Woods, 2014). Working memory is related to white matter integrity in many pathways, including the superior parietal lobule pathway, the medial temporo-frontal pathway, the uncinate fasciculus, the frontoparietal fasciculus, and the cingulum (Charlton, Barrick, Lawes, Markus, & Morris, 2010). A predilection for white-matter pathways may help explain how chemotherapy exerts its influence on working memory.

Neuroimaging

Neuroimaging methods have been used to better characterize the extent of chemotherapy-related cognitive dysfunction, and to explore its candidate mechanisms. In the last decade, evidence has been accumulating that frontal and parietal regions, along with subcortical white matter structures, may be vulnerable to structural and functional abnormalities following chemotherapy. This section will provide a brief introduction to some of the non-invasive neuroimaging techniques used to explore the neurobiological correlates of CRCI, and provide a review of the findings to date.

Magnetic Resonance Imaging. Magnetic resonance imaging (MRI) is a technology that permits investigators to directly observe internal anatomy with fine detail by manipulating the magnetic properties of certain protons. This technology can be used to study the structure and function of the human brain without exposing patients to radiation. Hydrogen atoms are abundant in the brain, and possess the nuclear magnetic resonance property - a required characteristic for MRI. When placed in a uniform external magnetic field, the protons will align themselves in parallel with the main magnetic field in either a low- or high-energy state. In MRI, radiofrequency pulses are applied that tip the alignment of the protons. When the radiofrequency signal is turned off, the spins begin to relax and realign with the main magnetic field. During the realignment process, detector coils capture energy emitted by protons, and the signal is then converted to images by a computer. When interpreted by specialized software, data gleaned from MRI can generate 2- and 3-D images of different tissue types – white matter, grey matter, cerebrospinal fluid – based on their different rates of relaxation and recovery (Huettel, Song, & McCarthy, 2009).

Functional magnetic resonance imaging. Functional magnetic resonance imaging (fMRI) takes advantage of the same physical properties and employs the same scanner as used

for structural MRI. Where MRI is used to create high-resolution images of anatomical structures, fMRI refers to the acquisition of imaging data used to make inferences about the presumed underlying neuronal activity that drives the changes in MRI signals. Images contain values of the signals related to neuronal hemodynamic responses. In this way, fMRI can be used as a proxy for neuronal activity. The metabolic needs of neurons are increased during neural activity, and to satisfy these demands, an increase in blood flow transports energy to active neurons. This increase in blood flow replaces deoxyhemoglobin molecules with oxygenated molecules in the active regions, resulting in a change of local magnetic signal (Huettel et al., 2009). As a result of this phenomenon - the blood oxygen level dependent (BOLD) effect - fMRI researchers can study the brain in action.

Neuroimaging and CRCI

In the last decade, neuroimaging studies of CRCI have emerged, generally supporting the findings from the neuropsychological studies of chemotherapy and cognition. Such studies have explored the integrity of white and grey matter, functional activations in response to cognitive tasks, and the efficiency of brain networks. In many cases, abnormal findings in patients exposed to chemotherapy have been related to decreases in performance on neuropsychological measures. Another line of evidence comes from the course of brain disruption, which often mirrors the trajectory of cognitive function. Specifically, from longitudinal neuropsychological and neuroimaging studies, there is accumulating evidence that disruption is most pronounced during and shortly after chemotherapy, with some recovery noted within one year after treatment. In the following sections, findings of CRCI neuroimaging studies of brain structure, function, and networks will be summarized.

Structural studies. Cross-sectional studies have reported white matter tract compromise in breast cancer patients shortly after chemotherapy (Abraham et al., 2008) and decades after treatment (de Ruiter et al., 2012; Koppelmans et al., 2014; Stouten-Kemperman et al., 2015). One of the first prospective studies of white matter integrity found post-treatment irregularities in the corpus callosum, and frontal and parietal regions that were related to performance in attention and verbal memory for breast cancer patients relative to chemotherapy-untreated patients (Deprez et al., 2012). A baseline comparison between these groups revealed no significant differences in white matter.

Studies of grey matter integrity have been more prominent in the CRCI literature (Conroy, McDonald, Ahles, West, & Saykin, 2013; Hakamata et al., 2007; Hosseini et al., 2012; Inagaki et al., 2007; Koppelmans et al., 2014; McDonald et al., 2010; McDonald, Conroy, Smith, et al., 2012; Scherling, Collins, MacKenzie, et al., 2012), and point to a course of grey matter alterations that are most pronounced following chemotherapy relative to baseline. With respect to post-treatment effects, cross-sectional studies have reported long-term grey matter abnormalities in chemotherapy-treated breast cancer patients up to 9.5 years after treatment (de Ruiter et al., 2012) and 21 years after chemotherapy (Koppelmans et al., 2012); however, one cross-sectional study found no CRCI related grey matter attenuation nearly 4 years after treatment (Inagaki et al., 2007). The first prospective voxel-based morphometry study of the CRCI population found no pre-chemotherapy structural differences when comparing patients to controls (McDonald et al., 2010). One month following treatment, the chemotherapy-treated group displayed broad grey matter alterations that partially recovered one year following exposure. In light of these mixed findings regarding the course of grey matter following treatment, Conroy et al. (2013) reported that grey matter density in the right superior and middle

frontal gyri was associated with post-chemotherapy interval, and that overall neuropsychological performance was related to mean grey matter density in those areas.

Thus, although few investigations of white matter have been undertaken in the chemotherapy and cognition field, those that have been conducted, along with those studying grey matter, offer similar findings to those from neuropsychological investigations of CRCI. Specifically, studies of brain structure integrity following chemotherapy suggest some breast cancer patients are susceptible to its neurotoxic effects, particularly in the short-term following treatment, with some patients experiencing recovery over time. Further, both grey and white matter compromise have been observed largely in frontal, parietal, and temporal regions. These findings support the reported neuropsychological deficits in domains largely subserved by these regions, including executive function, working memory, information processing speed, visuospatial ability, attention and concentration, motor functioning, and memory (Wefel et al., 2008).

Functional Studies. Functional neuroimaging studies have revealed neural activation differences between chemotherapy-treated breast cancer patients and chemotherapy-unexposed controls, both cross-sectionally and prospectively. With the exception of a positron-emission study by Silverman et al. (2007), functional neuroimaging studies of CRCI in breast cancer have employed fMRI, due in part to its non-invasive ability to provide a high quality, *in vivo* measure of neuronal activity with high spatial resolution (Askren et al., 2014; Cimprich et al., 2010; Conroy et al., 2012, 2013; de Ruiter et al., 2011; Ferguson et al., 2007; S R Kesler et al., 2011; Kesler, Bennett, Mahaffey, & Spiegel, 2009; López Zunini et al., 2013; McDonald, Conroy, Ahles, et al., 2012; Saykin et al., 2006; Scherling et al., 2011; Scherling, Collins, Mackenzie, et al., 2012; Stouten-Kemperman et al., 2015).

Functional irregularities between chemotherapy-treated breast cancer patients and controls have been found during tasks of executive function (de Ruiter et al., 2011; Kesler et al., 2011) and short-term verbal memory (de Ruiter et al., 2011; Kesler et al., 2009; López Zunini et al., 2013; Silverman et al., 2007). Both hyper- and hypoactivations have been predominantly circumscribed to frontal, temporal, and parietal regions. A cross-sectional study by de Ruiter et al., (2011) found decreased frontal and parietal activation during a planning task and decreased frontal, temporal, and parietal activation during a paired-association task approximately 10 years after chemotherapy exposure. These patterns were later related to decreases in white matter integrity and grey matter volume (de Ruiter et al., 2012). Hyporesponsiveness has also been reported in frontal regions, including the bilateral superior and middle frontal gyri, during a verbal declarative memory encoding task (Kesler et al., 2009). Similarly, frontotemporal hypoactivations have been reported during a verbal recognition task (López Zunini et al., 2013).

In keeping with findings from neuropsychological studies of CRCI that show working memory to be especially vulnerable to chemotherapy (Stewart et al., 2006), numerous functional neuroimaging studies of CRCI have focused on the neural underpinnings of this cognitive ability (Cimprich et al., 2010; Conroy et al., 2012, 2013; Ferguson et al., 2007; McDonald, Conroy, Ahles, et al., 2012; Saykin et al., 2006; Scherling et al., 2011). The *n*-back task is a common paradigm used to engage and assess working memory (Owen, McMillan, Laird, & Bullmore, 2005); it entails sequentially presenting stimuli, such as letters, and requiring the participant to respond when a stimulus that was presented *n* times previously is presented again. The *n*-back task is popular in investigations of working memory related brain activity due to its robust recruitment of brain regions associated with working memory, including the dorsolateral

prefrontal cortex, lateral premotor cortex, frontal poles, and medial and lateral posterior parietal cortices (Owen et al., 2005).

In the CRCI literature, the *n*-back task has been used to demonstrate frontoparietal working memory related neural activation differences between chemotherapy-treated breast cancer patients and controls (Conroy et al., 2012, 2013; Ferguson et al., 2007; McDonald, Conroy, Ahles, et al., 2012; Saykin et al., 2006). Using a two-person monozygotic twin study, Ferguson et al. (2007) were among the first to show a relationship between chemotherapy exposure and expansive frontal and parietal hyperactivations during performance of the *n*-back task, relative to the activation profile gleaned from the chemotherapy-free twin. A follow-up prospective study of working memory by McDonald et al. (2012) found that shortly after treatment, chemotherapy-treated breast cancer patients had attenuated medial and inferior frontal activations compared to pre-treatment baseline and one-year follow-up. Between groups analyses in that study revealed baseline hyperactivations in frontal and parietal regions on the part of the chemotherapy-exposed group that were attenuated one month following treatment, but were again hyperactive one year after chemotherapy. Despite neural activation differences, patients and controls appear to perform similarly with respect to reaction times, omissions, and correct responses (Ferguson et al., 2007; McDonald, Conroy, Ahles, et al., 2012). It has been suggested that activation differences, coupled with equivocal between-group task performance, are indicative of a compensatory mechanism whereby cognitive function is preserved in breast cancer patients, despite changes in neural activation and brain integrity (McDonald, Conroy, Ahles, et al., 2012; Scherling & Smith, 2013).

Although a large proportion of CRCI neuroimaging studies have investigated working memory, the relationship between chemotherapy and working memory related neural activity

remains unclear. First, only two studies have prospectively investigated working memory neural signatures (Conroy et al., 2013; McDonald, Conroy, Ahles, et al., 2012). Next, prior to beginning treatment, breast cancer patients can display activation abnormalities during working memory tasks (Cimprich et al., 2010; McDonald, Conroy, Ahles, et al., 2012; Scherling et al., 2011). It has been proposed that pre-treatment working memory dysfunction in newly diagnosed breast cancer patients can reflect fatigue, stress, and anxiety (Cimprich et al., 2010; Scherling et al., 2011), both of which can have a detrimental impact on working memory capacity (Chee et al., 2006; Shackman et al., 2006). Thus, further investigation is required, given the importance of one's ability to hold information online, and that this function over others appears to be the most negatively impacted by chemotherapy.

Brain network studies. In block-design fMRI studies, temporal correlations among brain regions activated during a task condition can imply neural network associations. The analytical approach to identify these temporal correlations has been labelled *functional connectivity* (Friston & Buchel, 2003). A recent longitudinal pilot study explored functional connectivity in nine breast cancer survivors as they performed the *n*-back task (Dumas et al., 2013). Decreased connectivity in the dorsal attention network was observed one month following chemotherapy; however, levels returned to baseline one year post-chemotherapy. In contrast, default mode network connectivity showed persistent decreased connectivity at the one-month and one-year post-chemotherapy intervals. Disrupted default mode network connectivity at rest has been found to discriminate chemotherapy-exposed breast cancer patients from their unexposed counterparts (Kesler et al., 2013), providing further support that dysfunctional networks may contribute to CRCI. Taken together, the results from the few existing fMRI studies of the breast cancer

population underscore the need for increased longitudinal investigations and further elucidation of the underlying functional correlates of CRCI.

Study Rationale

Neuropsychological sequelae have been documented in broad areas of cognition following chemotherapy administration, with working memory particularly susceptible to the injurious effects of chemotherapy (Stewart et al., 2006). Cognitive decline is related to both low and high dose regimens (Jenkins et al., 2006; van Dam et al., 1998), and has been found to have a dose-response relationship with chemotherapy (Collins et al., 2013).

Neuroimaging has been used increasingly in the last decade to characterize the neurobiological underpinnings of CRCI. In addition to altered white matter profiles, widespread grey matter volume reductions following chemotherapy have been noted, suggesting that the impact of chemotherapy is non-specific. Compromised grey matter has been found in medial temporal structures (e.g. hippocampus, parahippocampal gyrus; (Bergouignan et al., 2011; Inagaki et al., 2007; Kesler et al., 2013)), bilateral frontal regions (Inagaki et al., 2007; McDonald et al., 2010; McDonald, Conroy, Smith, et al., 2012), cerebellum (de Ruiter et al., 2012; McDonald et al., 2010), and parieto-occipital areas (de Ruiter et al., 2012; Inagaki et al., 2007). Overall total brain volume reductions in the absence of focal grey and white matter losses in long-term breast cancer survivors have been reported (Koppelmans et al., 2012).

Few studies have examined the neuropsychological impact of grey matter loss in breast cancer patients. Grey matter density in the right anterior frontal cortex in chemotherapy-treated breast cancer patients has been positively related to time-since-treatment, and overall neuropsychological performance (Conroy et al., 2012). Subjective executive function performance has also been related to post-treatment frontal grey matter volume reductions

(McDonald, Conroy, Smith, et al., 2012). Thus, although there is converging evidence that both cognition and brain matter are impacted similarly by chemotherapy, the extant literature on the topic is scarce. More work is needed to characterize the relationship between grey matter loss and cognition in chemotherapy-treated breast cancer patients.

In contrast to non-specific structural brain insults following chemotherapy, functional neuroimaging studies have predominantly revealed aberrant activation signatures in frontal and parietal regions. Post-treatment, executive function tasks have elicited hypoactivations in the dorsolateral prefrontal and posterior parietal cortices (de Ruiter et al., 2011; Kesler et al., 2011). Similarly, engagement in working memory tasks has been associated with hyperactivity in inferior and broad frontal areas, as well as in the parietal cortex (Ferguson et al., 2007; McDonald, Conroy, Ahles, et al., 2012). Irregular parietal and frontal activations have also been found in response to tasks that engaged verbal memory (de Ruiter et al., 2011; Kesler et al., 2009; López Zunini et al., 2013; Silverman et al., 2007). Given that working memory is the cognitive domain that is most impacted following chemotherapy, and that it is subserved by frontoparietal circuitry (Barbey, Koenigs, & Grafman, 2013; Charlton et al., 2010), more work should be performed to elucidate the influence of chemotherapy on these networks.

Aims of the Thesis

This thesis had two primary purposes. The first was to describe the longitudinal relationship between grey matter alterations and cognitive function in chemotherapy-treated breast cancer patients. Although a limited number of CRCI studies have explored the course of grey matter, they studied the relationship of its attenuation with subjective neuropsychological functioning (McDonald et al., 2010; McDonald, Conroy, Smith, et al., 2012). Thus, this thesis attempted to extend the existing CRCI literature by incorporating the use of a robust

neuropsychological battery to better characterize CRCI and its relation to grey matter attenuation.

The second aim of this thesis was to prospectively investigate the neural basis of working memory, given that this cognitive domain and its brain substrates appear most vulnerable for breast cancer patients receiving chemotherapy. To accomplish the goals of the thesis, two studies were conducted, each with pertinent hypotheses.

Hypotheses

Study 1. Given that current voxel-based morphometry studies suggest that grey matter reductions are most pronounced soon after chemotherapy and partially resolve over time (Conroy et al., 2013; McDonald et al., 2010), it was hypothesized that breast cancer patients would have broadly reduced grey matter volumes following chemotherapy, and that some recovery would be observed one year after treatment. Specific regions hypothesized to display attenuated recovery included the bilateral prefrontal cortex, medial temporal lobes, and the inferior parietal lobule.

Secondly, since participants of this study are a subset of participants from a larger neuropsychological study (Collins et al., 2013) in which there was a dose-response decline of cognitive function, it is hypothesized that areas exhibiting grey matter loss would be related to cognitive dysfunction. This would be observed, particularly, in executive function and working memory.

Study 2. With previous research showing pre-treatment working memory related hyperactivations in breast cancer patients compared to controls (McDonald, Conroy, Ahles, et al., 2012), it was hypothesized that breast cancer patients would have baseline hyperactivity bilaterally in the dorsolateral prefrontal cortex, and the superior parietal regions. Further, it was

hypothesized that the hyperactivations would be most pronounced and widespread within those regions shortly following chemotherapy, and partially resolve one year after treatment.

A second aim of this study was to investigate the functional connectivity of the frontoparietal network, which consists of regions frequently disrupted by chemotherapy, including the dorsolateral prefrontal cortex, and the superior parietal lobule (Cole et al., 2013). It was hypothesized that the frontoparietal network would display the greatest disruption shortly after chemotherapy, relative to baseline and one year post-treatment.

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A Prospective Study of Grey Matter and Cognitive Functioning Alterations in Chemotherapy-Treated Breast Cancer Patients

Lepage, C., Smith, A. M[1], Moreau, J., Barlow-Krelina, E., Wallis, N., Collins, B.,

MacKenzie. J., & Scherling, C.

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Abstract

Purpose: Subsequent to chemotherapy treatment, breast cancer patients often report a decline in cognitive functioning that can adversely impact many aspects of their lives. Evidence has mounted in recent years indicating that a portion of breast cancer survivors who have undergone chemotherapy display reduced performance on objective measures of cognitive functioning relative to comparison groups. Neurophysiological support for chemotherapy-related cognitive impairment has been accumulating due to an increase in neuroimaging studies in this field; however, longitudinal studies are limited and have not examined the relationship between structural grey matter alterations and neuropsychological performance. The aim of this study was to extend the cancer-cognition literature by investigating the association between grey matter attenuation and objectively measured cognitive functioning in chemotherapy-treated breast cancer patients.

Methods: Female breast cancer patients (n = 19) underwent magnetic resonance imaging after surgery but before commencing chemotherapy, one month following treatment, and one year after treatment completion. Individually matched controls (n = 19) underwent imaging at similar intervals. All participants underwent a comprehensive neuropsychological battery comprising four cognitive domains at these same time points. Longitudinal grey matter changes were investigated using voxel-based morphometry.

Results: One month following chemotherapy, patients had distributed grey matter volume reductions. One year after treatment, a partial recovery was observed with alterations persisting predominantly in frontal and temporal regions. This course was not observed in the healthy comparison group. Processing speed followed a similar trajectory within the patient

group, with poorest scores obtained one month following treatment and some improvement evident one year post-treatment.

Conclusion: This study provides further credence to patient claims of altered cognitive functioning subsequent to chemotherapy treatment.

Patient reports of cognitive changes subsequent to chemotherapy exposure abound in the breast cancer population. Self-perceived deterioration in mental functioning can adversely impact work and family life for breast cancer survivors (Boykoff, Moieni, & Subramanian, 2009). Evidence of chemotherapy-related cognitive impairment (CRCI) in breast cancer patients has mounted in the last several decades, as both retrospective cross-sectional and prospective longitudinal neuropsychological studies have found varying degrees of cognitive underperformance in chemotherapy-exposed breast cancer patients (for a review, see (O'Farrell, MacKenzie, & Collins, 2013)). Executive functioning, processing speed, and memory are domains frequently identified as vulnerable to chemotherapy exposure in this population (Wefel & Schagen, 2012). Meta-analyses suggest that CRCI is subtle, may affect a subgroup of patients only, and that, for some, it is a transient phenomenon (Falleti, Sanfilippo, Maruff, Weih, & Phillips, 2005; Stewart, Bielajew, Collins, Parkinson, & Tomiak, 2006). CRCI appears to have the greatest influence on cognitive functioning immediately following treatment to six months post treatment (Jim et al., 2012). However, some studies have found mild impairment years beyond treatment (Ahles et al., 2002; Koppelmans et al., 2012), while others have found pre-chemotherapy impairment in the breast cancer population (Ahles et al., 2007; Wefel, Saleeba, Buzdar, & Meyers, 2010), hinting at other contributing factors including the disease itself and highlighting the need for prospective longitudinal study designs.

Neuroimaging studies of chemotherapy-exposed breast cancer patients have started to elucidate the neural underpinnings of CRCI (for reviews, see (McDonald & Saykin, 2013; Scherling & Smith, 2013)). Research into the neuroanatomical correlates of CRCI has employed voxel-based morphometry (VBM) to explore grey matter compromise in the breast cancer population (Conroy et al., 2012; de Ruiter et al., 2012; Hakamata et al., 2007; Hosseini,

Koovakkattu, & Kesler, 2012; Inagaki et al., 2007; Koppelmans et al., 2012; McDonald, Conroy, Ahles, West, & Saykin, 2010; McDonald, Conroy, Smith, West, & Saykin, 2012; Scherling, Collins, MacKenzie, et al., 2012; Yoshikawa, Matsuoka, Yamasue, et al., 2005; Yoshikawa, Matsuoka, Inagaki, et al., 2005). VBM is a technique that enables researchers to make voxel-by-voxel comparisons of images of segmented brain matter volumes between groups of participants in an automated and unbiased manner (Ashburner & Friston, 2000; Good et al., 2002). The breast cancer literature suggests that the course of grey matter loss is similar to the course of CRCI. An early, retrospective VBM study found prefrontal and temporal grey matter reductions in a chemotherapy-exposed group four months after exposure; however, these differences were not present when the same study was conducted on a larger group a mean of 4.2 years since chemotherapy exposure (Inagaki et al., 2007). Some studies have found grey matter abnormalities in breast cancer patients at approximately 9.5 years after treatment (de Ruiter et al., 2012) and 21 years after chemotherapy (Koppelmans et al., 2012), suggesting that a subset of breast cancer patients exposed to chemotherapy are vulnerable to long-term grey matter deficits after chemotherapy exposure. The first prospective VBM study to investigate chemotherapy-related brain matter changes in breast cancer patients found no pre-chemotherapy structural differences between breast cancer patients and healthy controls while conducting a whole-brain analysis (McDonald et al., 2010). One month following treatment, the chemotherapy-exposed group displayed distributed grey matter attenuation that partially recovered one year subsequent to treatment. That was the first study to demonstrate a pattern of grey matter attenuation consistent with the course of cognitive impairment reported in neuropsychological studies, warranting a replication and extension study examining the link between neuropsychological functioning and grey matter disruption in chemotherapy-treated breast cancer patients.

To date, only one VBM study has investigated the relationship between grey matter volume and the results of a comprehensive neuropsychological assessment (Conroy et al., 2012). In that retrospective study, grey matter density in the right superior and middle frontal gyri was positively correlated with post-chemotherapy interval. Furthermore, overall neuropsychological performance was positively related to mean grey matter density of these regions. In light of those important findings, and given the cross-sectional design of that study, there exists a need for an increase in longitudinal studies examining grey matter alterations and their relationship with neuropsychological functioning.

In the present study, we employed VBM to measure longitudinal differences in whole-brain grey matter in breast cancer patients exposed to chemotherapy and we examined the relationship of these grey matter alterations to performance on a comprehensive neuropsychological battery. The present work extends a preliminary study conducted by our group that compared pre-chemotherapy volumetric differences between breast cancer patients and healthy controls (Scherling, Collins, MacKenzie, et al., 2012). Given that current VBM studies suggest that grey matter reductions are most pronounced soon after chemotherapy and partially resolve over time (Conroy et al., 2012; McDonald et al., 2010), it was hypothesized that breast cancer patients would have broadly reduced grey matter volumes following chemotherapy and that some recovery would be observed one year after treatment. We further hypothesized that frontotemporal areas exhibiting grey matter loss would be related to cognitive dysfunction, based on two lines of evidence. First, participants of this study were a subset of participants from a larger neuropsychological study (Collins, MacKenzie, Tasca, Scherling, & Smith, 2013) that showed a dose-response decline of cognitive functioning. Secondly, previous studies have demonstrated grey matter loss in frontotemporal regions and functional studies (de Ruiter et al.,

2011; Ferguson, McDonald, Saykin, & Ahles, 2007; Kesler, Bennett, Mahaffey, & Spiegel, 2009; Kesler, Kent, & O'Hara, 2011; Lopez Zunini et al., 2013; McDonald, Conroy, Ahles, West, & Saykin, 2012) have shown abnormal activations in these areas during executive functioning and memory tasks.

Material and methods

Participants

Twenty-three early-stage breast cancer patients and 23 healthy controls matched on age, sex, and education were recruited from the Ottawa Hospital Regional Cancer Centre following patient surgery to remove the cancer, but before patient chemotherapy commencement. Two patients withdrew from the study after treatment. At one year post-treatment, one patient withdrew and another had a recurrence and was excluded from the study. Members of the control group were recruited either by patient nomination or via print and web-based advertisements. The final sample for this study consisted of 19 breast cancer patients and 19 healthy controls. The present sample is a subset of participants from a larger study in which 60 breast cancer patients and their matched controls underwent longitudinal neuropsychological assessment (Collins et al., 2013) with a portion (38%) agreeing to further participate in imaging studies. As part of a larger imaging study, participants performed fMRI tasks related to verbal memory retrieval, response inhibition, and working memory following the structural scan (López Zunini et al., 2013; Scherling, Collins, Mackenzie, Bielajew, & Smith, 2011, 2012).

Clinical and demographic characteristics, including chemotherapy regimens, are listed in Table 1. Inclusion criteria for both groups were: 1) female; 2) no previous history of cancer or chemotherapy; 3) between 18 and 65 years of age at diagnosis; 4) fluent in English; and, 5) minimum of grade 8 education. Potential participants were excluded due to the presence of any

of the following: 1) metastasis of disease beyond axillary lymph nodes, 2) neo-adjuvant chemotherapy treatment, 3) serious psychiatric illness, neurological illness, or substance abuse, 4) MRI incompatibilities (e.g. metal implants, claustrophobia). This study was approved by the Ottawa Hospital Research Ethics Board, and the University of Ottawa Research Ethics Board.

Neuropsychological assessment

Prior to chemotherapy, following each patient's chemotherapy cycle, and one year after treatment completion, patients underwent a pencil-and-paper neuropsychological test battery as well as a computerized cognitive test (CNS-Vital Signs (Gualtieri & Johnson, 2006, 2008)). The traditional neuropsychological tests (Benedict, 1997; Brandt & Benedict, 2001; Brown, 1958; Delis, Kaplan, & Kramer, 2001; Fischer, Jak, Kniker, Rudick, & Cutter, 2001; Rao, Leo, Bernardin, & Unverzagt, 1991; US Army, 1944; Wechsler, 1997), listed in Table 2, were selected to parallel the cognitive domains covered by the computerized test battery and on the basis of their previously-observed sensitivity to the effects of cancer treatments (Stewart et al., 2008), their established reliability and validity (Benedict, 1997; Brandt & Benedict, 2001; Delis et al., 2001; Lezak, Howieson, Loring, Hannay, & Fischer, 2004; Strauss, Sherman, & Spreen, 2006; Wechsler, 1997), and the recommendations from the International Cognition and Cancer Task Force (Wefel, Vardy, Ahles, & Schagen, 2011). To mitigate practice effects, raw neuropsychological patient data were converted to standardized scores based on the means and standard deviations of the control group. Four domain-specific cognitive summary scores were computed on rational and empirical grounds: Processing Speed, Working Memory, Verbal Memory, and Visual Memory. Further elaboration of the assessments and the methodology employed to create the cognitive domains used in this study is provided elsewhere (Collins et al.,

2013). Neuropsychological data obtained closest to, but not surpassing, MRI data acquisition were used for analysis.

Table 1
Demographic and Clinical Characteristics

	Patients (n = 19)	**Controls (n = 19)**	***p-value***
Age at baseline (years)	50.2 (8.6)	49.3 (9.0)	0.76
Education			0.66
High School	2	3	
College	8	8	
Undergraduate Degree	5	2	
Graduate Degree	4	6	
Menopausal status at baseline			0.82
Menstruating	8	9	
Perimenopausal	4	2	
Postmenopausal	7	8	
Cancer stage			
I	3	–	
II	13	–	
III	3	–	
Chemotherapy regimen			
FEC-D (six cycles)[1]	12	–	
FEC-D (five cycles)	1		
CD (four cycles)	4	–	
CDOX (four cycles)[2]	2	–	
Type of surgery			
Modified Radical MX	7	–	
Simple MX	1	–	
Segmental MX	3	–	
Lumpectomy	8	–	
Time between (days)			
Surgery to T1 MRI	49.9 (15.2)	–	
T1 MRI to chemo	6.2 (4.9)	–	
End chemo to T2 MRI	32.0 (15.3)	–	
T1 MRI to T2 MRI	128.8 (23.0)	127.0 (25.0)	0.81
T2 MRI to T3 MRI	406.16 (70.3)	449.7 (106.9)	0.15

Mean (SD) or count values are shown. Units are arbitrary unless otherwise specified. FEC-D: fluorouracil + epirubicin + cyclophosphamide + docetaxel; CD: cyclophosphamide + docetaxel; CDOX: cyclophosphamide + doxorubicin; MX: mastectomy. [1]Two cases with epirubicin and one case with bevacizumab; [2]one case with paclitaxel.

Table 2

Neuropsychological Battery Organized by Cognitive Domain

PROCESSING SPEED	WORKING MEMORY	VERBAL MEMORY	VISUAL MEMORY
Digit-Symbol Coding (Wechsler, 1997)	Digit Span (Wechsler, 1997)	Hopkins Verbal Learning Test-Revised (Brandt & Benedict, 2001)	Brief Visuospatial Memory Test-Revised (Benedict, 1997)
Symbol Search (Wechsler, 1997)	Letter-Number-Sequencing (Wechsler, 1997)	CNS-VS Verbal Memory Index (Gualtieri & Johnson, 2006, 2008)	CNS-VS Visual Memory Index (Gualtieri & Johnson, 2006, 2008)
Trail Making Test A & B (US Army, 1944)	Paced Auditory Serial Addition Task (Fischer et al., 2001; Rao et al., 1991)		
CNS-VS Processing Speed Index (Gualtieri & Johnson, 2006, 2008)	Auditory Consonant Trigrams Test (Brown, 1958)		
CNS-VS Reaction Time Index (Gualtieri & Johnson, 2006, 2008)	Controlled Oral Word Association Test (Delis, Kaplan, & Kramer, 2001)		
	CNS-VS Flexibility Index (Gualtieri & Johnson, 2006, 2008)		
	CNS-VS Working Memory Index (Gualtieri & Johnson, 2006, 2008)		

Magnetic Resonance Imaging

MRI data for the patient group were acquired at three time points, with patient data acquired in similar intervals: T1) after surgery but before chemotherapy, radiation, and/or anti-estrogen treatment; T2) approximately one month subsequent to chemotherapy regimen completion; and T3) approximately one year following chemotherapy.

All images were acquired with a 1.5 Tesla Siemens Magnetom Symphony MR scanner. A gradient echo localizer was acquired and used to prescribe a 3D FLASH (Fast Low Angle SHot) spoiled gradient sequence with the following parameters: TR = 629 ms, TE = 15 ms, field of view: 187 x 250 mm, flip angle: 90 degrees, acquisition matrix: 256 x 192, 5mm thick axial slices, voxel size 1 x 1 x 5 mm.

The 3D data were analyzed using FSL-VBM (Douaud et al., 2007), an 'optimized' VBM protocol (Good et al., 2002) implemented in FSL tools (Smith et al., 2004). The brain extraction tool BET (Smith, 2002), was used to remove skin and skull. Subsequently, the brain-extracted images were tissue-segmented and the grey matter partial volume images were registered to the MNI152 standard space using non-linear registration (Andersson, Jenkinson, & Smith, 2007). The registered images were averaged and flipped along the x-axis to create a symmetric, study-specific grey matter template in order to reduce the effect of inter-subject variability during registration. The native grey matter images were then linearily re-registered to this template and modulated (i.e. divided by the Jabobian of the warp field) to correct for local expansion or contraction due to the non-linear component of the spatial transformation. Smoothing with an isotropic Gaussian kernel with a sigma of 3mm was applied to the modulated grey matter images.

Next, within-group voxel-wise threshold-free cluster enhancement-based (Smith & Nichols, 2008) GLM analyses were conducted using permutation-based non-parametric testing with 5,000 permutations on whole-brain grey matter volumes. Statistical maps of within-group comparisons thresholded at $p < 0.01$ uncorrected for multiple comparisons were used to generate region of interest (ROI) masks. Uncorrected values were used as a means of selecting ROIs for further analysis. A composite whole-brain mask covering all regions of significant differences and 14 masks covering the intersection of these regions and anatomical ROIs were defined with the AAL atlas tool (Tzourio-Mazoyer et al., 2002) using WFU PickAtlas (Maldjian, Laurienti, & Burdette, 2004; Maldjian, Laurienti, Kraft, & Burdette, 2003) were created.

Region of Interest Analysis

Using R (R Development Core Team, 2014), ROI data were compared across time points with Welch's t-tests and *p* values were adjusted for multiple comparisons using the Benjamini-Hochberg procedure (Benjamini & Hochberg, 1995).

Neuropsychological and Demographic Data

Differences across neuropsychological scores were investigated with repeated measures ANOVA and Tukey pairwise comparisons. The relationship between grey matter volumes and neuropsychological performance within the patient group was examined with HLM7 (Raudenbush, Bryk, Cheong, & Condon, 2011) using a two-level hierarchical linear model (HLM; Raudenbush & Bryk, 2002) with time points nested within patients. Distinct HLM analyses were conducted in order to assess the correlation between each ROI and each cognitive domain. Welch's two sample t-tests were used to compare all demographic data, except in the case of nominal data where Fisher's exact tests were used.

Results

Sample Characteristics

Demographic characteristics are listed in Table 1. The patient group ranged in age from 35 to 64 years and the controls ranged in age from 31 to 61 years. The interval between scanning sessions did not differ between groups ($p > 0.05$, Table 1). For patients, T1 neuropsychological assessments were conducted on average 10.20 days ($SD = 8.12$) before chemotherapy, T2 neuropsychological assessments were conducted on average 17.75 days ($SD = 7.17$) after final chemotherapy exposure, and T3 neuropsychological assessments were completed on average 392.5 days ($SD = 46.77$) following T2 assessments. After T2 and before T3, 10 patients commenced hormonal therapy. Similarly, during this interval 13 patients underwent radiotherapy. Between T2 and T3, all patients that were either menstruating or perimenopausal at T1 became menopausal; however, this status did not change for controls.

Within-group grey matter changes

Table 3 shows the grey matter volume differences between scans for patients in the composite whole-brain mask and ROIs. At T2 relative to T1, patients showed a reduction of grey matter volume in frontal, temporal, parietal, and occipital regions (Figure 1). There were no areas of increased volume at T2 relative to T1. At one year after chemotherapy relative to T1, significant grey matter reductions were observed in bilateral frontal and temporal regions and all other reductions observed from T1 to T2 were no longer significant. Controls did not show any decrease from T1 to T2; however, they did display an unexpected increase in grey matter volume in the right amygdala from T1 to T2. At T3 relative to T1, this increase was no longer significant; yet, there was a significant increase in grey matter volume in the left lingual gyrus.

Table 3

Longitudinal Changes in Patient VBM Values

	Pre-chemo to 1-month post (n = 20)			Pre-chemo to 1-year post (n = 19)			1-month post to 1-year post (n = 19)		
Regions	Mean Δ	*T* value	*P* value	Mean Δ	*T* value	*P* value	Mean Δ	*T* value	*P* value
Composite Whole-Brain	-909.08 ± 162.64	-11.70	<0.001	-387.78 ± 164.58	-4.95	<0.001	535.88 ± 186.53	6.04	<0.001
Left Hemisphere									
Medial Orbitofrontal Gyrus	-5.85 ± 3.11	-3.94	0.003	-2.52 ± 3.59	-1.474	0.158	3.63 ± 4.17	1.83	0.130
Inferior Orbitofrontal Gyrus	-9.62 ± 4.29	-4.69	<0.001	-3.62 ± 4.75	-1.61	0.125	6.40 ± 4.29	3.13	0.008
Inferior Frontal Operculum	-26.21 ± 11.68	-4.70	<0.001	-15.70 ± 10.29	-3.20	0.007	12.38 ± 14.28	1.82	0.085
Middle Temporal Gyrus	-95.23 ± 40.65	-4.90	<0.001	-48.55 ± 74.59	-1.37	0.188	47.47 ± 65.50	1.52	0.152
Insular Cortex	-20.91 ± 8.48	-5.16	<0.001	-16.14 ± 11.29	-3.00	0.011	4.82 ± 11.29	0.90	0.381
Superior Temporal Gyrus	-79.35 ± 29.94	-5.55	<0.001	-40.30 ± 24.20	-3.50	0.004	42.86 ± 27.16	3.32	0.004
Anterior Cingulate	-15.49 ± 5.14	-6.30	<0.001	-8.87 ± 8.08	-2.31	0.033	5.83 ± 9.04	1.36	0.192
Calcarine Cortex	-3.70 ± 1.80	-4.41	<0.001	-0.43 ± 2.35	-0.39	0.703	3.11 ± -2.18	2.99	0.011
Right Hemisphere									
Middle Frontal Gyrus	-8.30 ± 3.70	-4.70	<0.001	-4.97 ± 3.26	-3.20	0.007	3.92 ± 4.52	1.82	0.085
Gyrus Rectus	-29.36 ± 12.97	-4.74	<0.001	-3.99 ± 21.89	-0.38	0.706	24.03 ± 20.51	2.46	0.036
Paracentral Lobule	-34.50 ± 14.91	-4.84	<0.001	-18.34 ± 21.67	-1.78	0.092	17.40 ± -16.53	2.21	0.060
Precuneus	-6.89 ± 2.61	-5.53	<0.001	-5.61 ± 5.84	-2.02	0.089	0.80 ± 6.17	0.27	0.788
Hippocampus	-11.41 ± 3.63	-6.58	<0.001	-7.89 ± 5.71	-2.91	0.014	3.01 ± 5.11	1.24	0.232
Anterior Cingulate	-11.20 ± 4.88	-4.80	<0.001	-4.99 ± 5.74	-1.82	0.100	5.42 ± 6.53	1.74	0.100

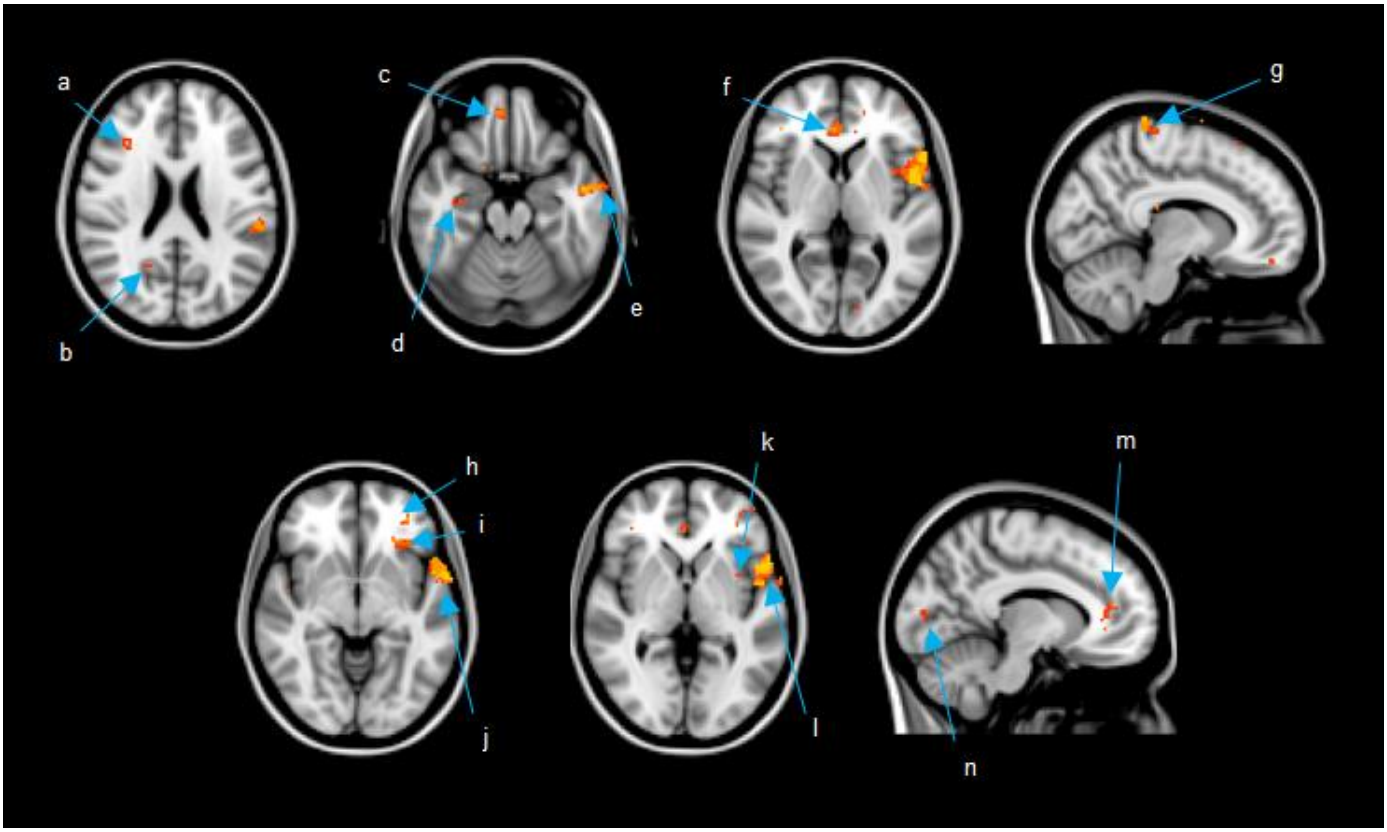

Figure 1. Images are presented in radiological view where right is the patient's left side and left is the patient's right side. Coordinates presented in MNI space. a) middle frontal gyrus (36 28 22); b) precuneus (20 -58 25); c) gyrus rectus (6 48 -20); d) hippocampus (34 -14 -20) e) middle temporal gyrus (-58 -4 -20); f) anterior cingulate (4 38 5); g) paracentral lobule (10 -36 75); h) medial orbitofrontal gyrus (-32 44 -5); i) inferior orbitofrontal gyrus (-32 28 -5); j) superior temporal gyrus (-65 2 -5); k) insular cortex (-36 6 0); l) inferior frontal operculum (-54 10 0); m) anterior cingulate (-10 38 10); n) calcarine cortex (-12 -86 5).

Cognitive Domain Scores

Patient mean scores are presented in Table 4. An analysis of variance for processing speed revealed a significant difference among the time points ($F(2, 35)= 14.59$, $p < 0.001$). A post hoc Tukey test showed that patients scored significantly better at baseline relative to T2 ($p < 0.001$) and T3 ($p = 0.004$). Although processing speed scores improved from T2 to T3, this difference was only marginally significant ($p = 0.094$). Scores on the three other cognitive domains degraded over time; however, the observed differences were not significant.

Table 4

Patient Within-Group Cognitive Domain Scores ANOVA

	Time1		**Time 2**		**Time 3**		**ANOVA**		**Tukey's HSD**		
	***n* = 19**		***n* = 19**		***n* = 19**		***df* = 2, 35**		**T1 to T2**	**T1 to T3**	**T2 to T3**
Domain	*M*	*SD*	*M*	*SD*	*M*	*SD*	*F*-ratio	*p* value	*p* value	*p* value	*p* value
Processing Speed	-0.05	1.03	-0.54	1.29	-0.43	1.19	14.59	<0.001	<0.001	0.004	0.094
Working Memory	-0.26	0.71	0.36	0.70	-0.35	0.72	0.80	0.457	0.421	0.887	0.726
Verbal Memory	-0.18	1.14	0.20	1.25	-0.64	1.44	2.40	0.105	0.993	0.121	0.154
Visual Memory	-0.08	0.93	-0.28	0.97	-0.39	1.11	1.39	0.262	0.416	0.259	0.942

Scores represent average z-scores on tests comprising each domain, referenced to the control group mean and standard deviation at the same time point

Cognitive functioning and relationship to grey matter volumes

Listed in Table 5 are the correlations between ROI grey matter volumes and the four cognitive domains. Processing speed displayed a positive relationship with the whole-brain composite ($r = 0.61$, $p < 0.001$) and frontal, temporal, and occipital areas. Working memory showed a positive relationship with the left medial orbitofrontal gyrus ($r = 0.51$, $p = 0.007$) and the right middle frontal gyrus ($r = 0.42$, $p < 0.05$). Visual memory was positively related to grey matter volume in the left inferior frontal operculum ($r = 0.71$, $p = 0.009$) and the right middle frontal gyrus ($r = 0.71$, $p < 0.009$). There was no relationship between grey matter in the ROIs and composite whole-brain with verbal memory.

Table 5

Patient whole brain and ROI grey matter volume correlations with cognitive domains

Region	Processing Speed			Working Memory			Verbal Memory			Visual Memory		
	r	t(18)	*p* value	r	t(18)	*p* value	r	t(18)	*p* value	r	t(18)	*p* value
Composite Whole-Brain	0.61	4.75	<0.001	0.01	0.09	0.933	0.05	0.16	0.877	0.18	0.85	.407
Left Hemisphere												
Medial Orbitofrontal Gyrus	0.36	4.27	<0.001	0.51	3.05	0.007	-0.35	-1.20	0.244	0.19	0.88	0.393
Inferior Orbitofrontal Gyrus	0.36	3.91	0.001	0.12	0.75	0.461	-0.63	-1.81	0.088	-0.18	-0.75	0.461
Inferior Frontal Operculum	0.54	3.15	0.006	0.42	2.11	0.049	0.48	1.25	0.229	0.71	2.91	0.009
Middle Temporal Gyrus	0.21	1.29	0.213	-0.02	-0.15	0.883	0.24	0.76	0.457	0.14	0.63	0.540
Insular Cortex	0.44	4.21	<0.001	-0.07	-0.43	0.672	-0.10	-0.27	0.789	0.05	0.21	0.837
Superior Temporal Gyrus	0.56	4.56	<0.001	0.17	0.01	0.948	0.09	0.29	0.775	0.05	0.22	0.829
Anterior Cingulate	0.35	2.25	0.037	0.12	0.91	0.376	0.04	0.11	0.917	0.31	1.39	0.180
Calcarine Cortex	0.26	2.55	0.020	-0.01	-0.09	0.929	0.15	0.48	0.640	0.13	-0.56	0.582
Right Hemisphere												
Middle Frontal Gyrus	0.54	3.15	0.006	0.42	2.11	0.049	0.48	1.25	0.229	0.72	2.91	0.009
Gyrus Rectus	0.14	0.94	0.359	0.09	0.56	0.582	0.15	1.25	0.229	0.32	1.50	0.150
Paracentral Lobule	0.58	3.86	0.001	0.14	-0.85	0.408	0.04	0.15	0.886	-0.02	-0.09	0.932
Precuneus	0.19	1.25	0.227	-0.04	-0.38	0.709	0.13	0.39	0.698	0.16	0.59	0.564
Hippocampus	0.26	1.95	0.066	0.10	0.88	0.393	-0.12	-0.40	0.696	-0.08	-0.36	0.721
Anterior Cingulate	0.01	0.09	0.933	0.07	0.56	0.585	-0.15	-0.46	0.653	0.13	0.57	0.577

Discussion

VBM analyses showed diffuse reductions in brain regions of breast cancer patients one month after chemotherapy. This attenuation recovered in nearly half of the regions one year post-chemotherapy. These results provide both evidence of a neural basis for CRCI and optimism for the recovery from the injurious effects that chemotherapy appears to have on the brain.

Our primary hypothesis that grey matter alterations would be more pronounced and distributed shortly after chemotherapy, and then partially resolve one year post-chemotherapy, was supported. Diffuse grey matter alterations in the patient group approximately one month after chemotherapy exposure are congruent with existing VBM studies that have shown distributed grey matter disruption in the breast cancer population shortly after chemotherapy treatment (Inagaki et al., 2007; McDonald et al., 2010; McDonald, Conroy, Smith, et al., 2012). Additionally, our results support the converging evidence from both structural (Inagaki et al., 2007; McDonald et al., 2010; McDonald, Conroy, Smith, et al., 2012) and functional studies (de Ruiter et al., 2011; Kesler et al., 2009, 2011; Lopez Zunini et al., 2013; McDonald, Conroy, Ahles, et al., 2012; Silverman et al., 2007) that the frontal lobes appear particularly sensitive to chemotherapy. These findings are important in light of common reports of acute executive function and working memory difficulties in patients subsequent to chemotherapy (Wefel & Schagen, 2012) because these cognitive functions are subserved by the frontal lobes (Fletcher & Henson, 2001). Our findings of both attenuated grey matter volume in the frontal lobes and the positive relationship between GM reduction in these regions with poorer performance on executive function, working memory, and visual memory strengthen the neuroanatomical evidence of CRCI.

In contrast to one month post-chemotherapy, fewer regions displayed reduced GM at one year post-treatment relative to baseline. Regions displaying persistent grey matter loss remained bilaterally distributed in frontotemporal regions. Enduring frontal grey matter suppression was found in the left anterior cingulate gyrus, left inferior frontal operculum, and right middle frontal gyrus, congruent with a previous report of chronic frontal insult in chemotherapy-treated breast cancer patients (McDonald et al., 2010). The region of the left superior temporal gyrus and left insula that had pronounced reduction at T2 relative to T1 (see Figure 1) did not fully resolve. The superior temporal gyrus and insula share efferent and afferent connections (Flynn, 1999) and this may help explain the concomitant insults to these regions observed in the present study. Interestingly, the right hippocampus displayed reduced grey matter, concordant with previous studies that have demonstrated prolonged hippocampal compromise (Bergouignan et al., 2011; Kesler et al., 2013; McDonald et al., 2010), but in contrast to a study by Yoshikawa et al. (2005) that failed to find hippocampal insult.

The diffuse nature of grey matter modulation observed in this and similar studies, along with some of the regional volume loss discordance across extant grey matter studies of chemotherapy in breast cancer, may be attributable to the inclusion of heterogeneous chemotherapy regimens. Although nearly all cytostatic agents have been associated with neurobiological effects, the mechanisms and outcomes vary across treatments (for a review, see (Seigers, Schagen, Van Tellingen, & Dietrich, 2013)). Some chemotherapeutic agents, such as methotrexate, 5-fluorouracil, and cyclophosphamide, appear to have direct cytotoxic effects via their ability to penetrate the blood-brain-barrier (BBB; Dietrich, 2010); however, other agents appear to have indirect effects due to their inability to cross the BBB. A recent study by Kesler et al. (2013) suggests that elevated pro-inflammatory cytokine expression, as seen in BBB

impermeable agents like doxorubicin, may have direct and indirect injurious effects on brain structures. Our present study included both BBB permeable and impermeable chemotherapeutic agents (see Table 1). It will be important for future studies to tease apart the differential effects of various chemotherapy treatments on the brain.

Our secondary hypothesis that grey matter attenuation would be related to cognitive functioning was supported. Although processing speed was positively related to distributed grey matter volumes, the association was observed predominantly in frontotemporal regions. This included the left insula and a portion of prefrontal areas with which the insula shares bidirectional connections (Flynn, 1999), specifically, the left medial and inferior regions of the orbitofrontal cortex (OFC) and the left inferior frontal operculum. The insula is critical for neural communication between the prefrontal cortex and more posterior regions (Augustine, 1996), and disruption to the insula and associated regions may underlie some of the cognitive difficulties expressed by chemotherapy-exposed breast cancer patients.

Interestingly, processing speed and working memory were positively correlated with grey matter volume in the medial orbitofrontal gyrus. This is a notable finding given the clinical implications. Previous studies have associated the OFC with a range of cognitive processes including decision-making (Plassmann, O'Doherty, & Rangel, 2010), emotion (Rolls & Grabenhorst, 2008), and response inhibition (Horn, Dolan, Elliott, Deakin, & Woodruff, 2003). Grey matter loss in this region of the OFC was significant for patients between pre-chemotherapy exposure and one month post-treatment, consistent with the pattern of decreased cognitive performance during the same interval. Our findings underscore the extensive impact that exposure to chemotherapy may have on breast cancer patients. In light of our results, work showing that the OFC is involved in processes beyond the cognitive domains selected in our

study points to a potential for chemotherapy exposure to adversely impact the lives of breast cancer patients more widely than suggested by our results alone.

The strengths of this study include its longitudinal design and the administration of a comprehensive battery of objective neuropsychological measures that covered a broad range of cognitive processes. We acknowledge that there are limitations to our study that necessitate a degree of caution when interpreting our findings. Primarily, we did not include a chemotherapy-naïve comparison group and, consequently, we could not control for the potential influence of cancer-related factors. Existing VBM studies that have included both a healthy control group and a chemotherapy-naïve breast cancer control group have found no within-group grey matter differences in these control groups in contrast to the decline observed in chemotherapy-exposed breast cancer patients (McDonald et al., 2010; McDonald, Conroy, Smith, et al., 2012), suggesting that grey matter alterations may stem from chemotherapy exposure.

The number of treatment cycles and types of surgery varied across breast cancer patients (see Table 1). Given our limited sample size, we could not control for the influence of these potentially confounding factors. Currently, one cross-sectional study has examined the effects of a heterogeneous chemotherapy regimen on grey matter volumes in a large sample (Koppelmans et al., 2012); it will be important for future longitudinal investigations to employ a similar approach. Treatment-induced menopausal symptoms co-occur with cognitive impairment following chemotherapy in breast cancer patients (Fan et al., 2005). Although our patients and controls were closely matched at baseline, at T3 relative to T2 all patients were menopausal while controls remained unchanged. As a result, we were unable to control for the effects of menopausal status. From time T2 to T3, some patients received radiotherapy or commenced hormonal therapy. Reports in the literature suggest that these treatments may perturb cognition

(Bender, Paraska, Sereika, Ryan, & Berga, 2001; Quesnel, Savard, & Ivers, 2009). However, despite their administration, an overall improvement in GM volumes was observed from T2 to T3, suggesting that these therapies may have had a negligible effect.

In summary, the present study demonstrated grey matter volume loss in diffuse brain regions in breast cancer patients one month following chemotherapy treatment. One year following treatment, grey matter was partially recovered. Grey matter volumes were related to cognitive performance in the domains of processing speed, working memory, and visual memory. Cognitive dysfunction was found to follow a similar course to grey matter changes, particularly in the domain of processing speed. This study strengthens the evidence for the relationship between brain alterations and objectively measured cognitive difficulties in breast cancer patients exposed to chemotherapy. In addition to the burden of being diagnosed with a life-threatening disease, breast cancer patients must contend with potential adverse side effects of treatment. Cognitive and neurophysiological alterations touch many areas of survivors' lives, warranting future research to further elucidate the mechanisms of CRCI and to improve breast cancer patients' quality of life.

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Post-chemotherapy recovery of working memory brain activity and functional connectivity in breast cancer: a prospective fMRI study

Chris Lepage, Carole Scherling, Nancy Wallis, Jeremy T. Moreau, Miranda Kiyomi Setoguchi, Zahra Mawani, Joyce MacKenzie, Barbara Collins, and Andra Smith

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Abstract

Chemotherapy-related cognitive impairment (CRCI) has been demonstrated across various cognitive domains subserved by frontoparietal brain regions. Working memory appears to be particularly impacted in the short term; yet, the longitudinal course of dysfunction in this cognitive domain remains to be fully explored. Neuroimaging investigations of the underlying networks involved in CRCI are emerging, but much work remains to elucidate the affected circuitry. The aim of this study was to prospectively examine working memory-related functional connectivity in the frontoparietal network (FPN) of newly diagnosed breast cancer patients (n = 23) and cancer-free controls (n = 23). Women were recruited to complete a working memory task during functional magnetic resonance imaging before patient chemotherapy, one month after therapy, and one year after treatment cessation. A seed in the dorsolateral prefrontal cortex was used to assess functional connectivity within the FPN. Between-group differences were present at one month after chemotherapy only, with patients displaying broad hyperactivity in the frontal, parietal, and occipital regions while performing a working memory task. Similarly, patients had reduced FPN connectivity between the left dorsolateral prefrontal cortex and a left occipito-parietal region. This is the first CRCI neuroimaging study to show a return to baseline levels of brain activity in chemotherapy-treated breast cancer patients. This study adds to the accumulating evidence of acute post-chemotherapy cognitive sequelae, and offers a neurofunctional account of the rebound effect of working memory impairment reported in the neuropsychological literature of CRCI.

The advancement of treatment for breast cancer has resulted in an increase in survivorship accompanied by a growing concern for the impact on cognition from adjuvant therapies. Chemotherapy exposure is commonly associated with subjective reports of diminished cognition in breast cancer patients (e.g., see (Pullens, De Vries, & Roukema 2010)). Although the concordance between subjective cognitive complaints and the results of objective testing is limited (Ganz et al. 2013; Pullens et al. 2010), an accumulating body of literature supports the existence of objective cognitive decline over the course of treatment of breast cancer (for a review, see (Wefel & Schagen 2012)).

Chemotherapy-related cognitive impairment (CRCI) is most pronounced during and shortly after treatment, relative to baseline and long-term follow-up (Collins, MacKenzie, Tasca, Scherling, & Smith 2013; Collins, Mackenzie, Tasca, Scherling, & Smith 2014; Jansen, Cooper, Dodd, & Miaskowski 2011; Wefel, Lenzi, Theriault, Davis, & Meyers 2004), It has been reported across a breadth of cognitive domains, including memory, attention, executive function, and processing speed; however, the extent and frequency of the impairment across cognitive domains is variable (Ahles et al. 2002; Hermelink et al. 2007; Hurria et al. 2006; Jansen et al. 2011; Quesnel, Savard, & Ivers 2009; Schagen, Muller, Boogerd, Mellenbergh, & van Dam 2006; Wefel, Saleeba, Buzdar, & Meyers 2010). A number of studies have found that working memory may be particularly sensitive to the injurious effect of chemotherapy (Bender et al. 2006; Collins, Mackenzie, Stewart, Bielajew, & Verma 2009; Shilling, Jenkins, Morris, Deutsch, & Bloomfield 2005; Stewart et al. 2008; Stewart, Bielajew, Collins, Parkinson, & Tomiak 2006) suggesting that this cognitive domain should be a focus in studies that seek to better characterize CRCI.

Neuroimaging has been used increasingly to investigate the neural mechanisms underlying CRCI. Retrospective and prospective studies have reported brain structure (Abraham et al., 2008; Conroy et al., 2012; de Ruiter et al., 2012; Deprez et al., 2011, 2012; Inagaki et al. 2007; Koppelmans et al., 2011; Lepage et al., 2014; McDonald, Conroy, Ahles, West, & Saykin, 2010; McDonald, Conroy, Smith, West, & Saykin, 2012; Scherling, Collins, MacKenzie, et al., 2012) and function (Askren et al., 2014; Berman et al., 2014; Cimprich et al., 2010; de Ruiter et al., 2011; Ferguson, McDonald, Saykin, & Ahles, 2007; Kesler, Bennett, Mahaffey, & Spiegel, 2009; Kesler, Kent, & O'Hara, 2011; López Zunini et al., 2013; McDonald, Conroy, Ahles, West, & Saykin, 2012; Scherling, Collins, Mackenzie, Bielajew, & Smith, 2011, 2012; Silverman et al., 2007) irregularities in breast cancer patients that generally follow the course of impairment reported by studies of cognitive function. Although abnormal neural activation has been reported across studies of various cognitive abilities - including executive function, memory, and attention - working memory has been most commonly studied (for reviews, see (McDonald & Saykin, 2013; Scherling & Smith, 2013)). Atypical activation patterns are most typically seen in frontal and parietal regions in breast cancer patients (Conroy et al. 2012; de Ruiter et al., 2011; Ferguson et al., 2007; Kesler et al., 2011; McDonald, Conroy, Ahles et al., 2012). In addition to reflecting the neural demands of the tasks, these activation profiles may reveal common brain networks that are particularly vulnerable to the effects of chemotherapy.

Studies of neural networks in breast cancer patients are few; however, emerging evidence points to disrupted global brain network organization in chemotherapy-treated breast cancer patients (Bruno, Hosseini, & Kesler, 2012; Hosseini, Koovakkattu, & Kesler, 2012). In a recent longitudinal pilot study that used a working memory task, reduced functional connectivity was found in the dorsal attention network one month after chemotherapy relative to baseline, which

partially recovered one year later (Dumas et al., 2013). In the same study, the authors also reported persistent decreased functional connectivity in the default mode network that began one month post-chemotherapy. These findings highlight the importance of combining emerging analysis methods and common assessments to better characterize suspected cognitive deficits.

The frontoparietal network (FPN) is a cognitive and action control system that flexibly recruits and updates neural hubs in order to guide adaptive behaviour across a range of cognitive demands (Cole et al., 2013; Koziol, Barker, Joyce, & Hrin, 2014). Hubs within the FPN have been uniquely associated with cognitive control and working memory (Harding, Yücel, Harrison, Pantelis, & Breakspear, 2014). Thus, considering the irregular neural activation and network profiles within frontoparietal regions of chemotherapy-treated breast cancer patients, the investigation of the FPN may offer greater insight into the neural substrates of working memory deficits in this population.

The purpose of this study was to prospectively compare functional connectivity within the FPN during a task of working memory between breast cancer patients and healthy controls. It was hypothesized that breast cancer patients would show reduced functional connectivity shortly after treatment, and that there would be recovery one year later. A secondary aim of this study was to replicate findings of abnormal neural activation during a commonly studied working memory paradigm in the CRCI neuroimaging literature.

Methods

Participants

Twenty-three early-stage breast cancer patients and 23 healthy controls matched on age, sex, and education were recruited via the Ottawa Hospital Regional Cancer Centre from a pool of candidates participating in a prospective study of the effects of chemotherapy on cognition

(Collins et al. 2013). Patients were recruited after surgery but before commencing chemotherapy, radiation, or hormone therapy. Each patient nominated her own control; when she was unable to do so, a control was recruited through posters and internet advertisements. All participants were required to be between the ages of 18 and 65 years, to be fluent in English, to possess at least a grade-8 education, and to reside within 30-miles of Ottawa. Additional exclusion criteria were: previous history of cancer or chemotherapy, psychiatric or neurological illness, substance abuse, and MRI contraindications (e.g. metal implants). Specific to the breast cancer group, patients with metastasis of disease beyond axillary lymph nodes were excluded. Various chemotherapy regimens were accepted (Table 1). Two patients withdrew from the study after treatment onset. At one year post-treatment, an additional patient withdrew and another was excluded from the study due to a recurrence.

Assessment Protocol and Schedule

Neuropsychological measures were collected from the patients after surgery, but before chemotherapy (Time 1), following each chemotherapy cycle, and one year following completion of chemotherapy (Time 3). A detailed description of each testing session is provided elsewhere (Collins et al. 2013). MRI measures were collected at Time 1, one month after the last chemotherapy cycle (Time 2), and Time 3. Psychometric testing and MRI scanning sessions for controls were yoked to a respectively matched patient.

MRI acquisition protocol

Imaging data were acquired using a 1.5 Tesla Siemens Magnetom Symphony MRI scanner. A gradient echo localizer was acquired and used to prescribe a subsequent 3D Fast Low Angle Shot spoiled gradient sequence, with TR/TE 22/9.2ms, flip angle 30º, field of view 256x256 mm. Whole brain echo planar fMRI based on the blood oxygen level-dependent effect

was performed using a gradient echo pulse sequence (TR/TE 3000/40ms, flip angle 90º, field of view: 250x187.5,mm 64x64 matrix, slice thickness 5mm, 27 axial slices, bandwidth 2430 Hz per pixel). As part of the imaging protocol, participants performed a total of four tasks aimed to investigate different cognitive processes. The results of these investigations and studies of structural data acquired during the imaging sessions have been published separately (Lepage et al., 2014; López Zunini et al., 2013; Scherling et al., 2011; Scherling, Collins, Mackenzie, et al., 2012; Scherling, Collins, MacKenzie, et al., 2012).

Table 1
Baseline Demographic and Clinical Characteristics

	Patients (n = 23)	**Controls (n = 23)**	***p-value***
Age at baseline (years)	51.5 (8.6)	50.4 (8.8)	.69
Education			
High School	2	3	.05
College	11	12	
Undergraduate Degree	6	2	
Graduate Degree	4	6	
Menopausal status at baseline			.68
Menstruating	8	9	
Perimenopausal	4	2	
Postmenopausal	11	12	
Cancer stage			
I	4	–	
IIa	10	–	
IIb	5	–	
III	4	–	
Chemotherapy regimen[1]			
FEC-D (six cycles)[2]	14	–	
FEC-D (five cycles)	2	–	
CD (four cycles)	5	–	
CDOX (four cycles)	1	–	
Time between (days)			
Surgery to T1 MRI	49.8 (15.3)	–	
T1 MRI to chemotherapy	6.0 (4.5)	–	
End chemo to T2 MRI	31.4 (14.7)	–	
T1 MRI to T2 MRI	128.8 (23.0)	127.0 (25.0)	.81
T2 MRI to T3 MRI	406.16 (70.3)	449.7 (106.9)	.15

Note. Mean (SD) or count values are shown. Units are arbitrary unless otherwise specified. [1] Data missing for one patient. FEC-D: fluorouracil + epirubicin + cyclophosphamide + docetaxel; CD: cyclophosphamide + docetaxel; CDOX: cyclophosphamide + doxorubicin + paclitaxel; [2]three cases with trastuzumab and one case with bevacizumab.

fMRI task

The n-back task is commonly used to study working memory in both the breast cancer neuroimaging literature and in other imaged populations (for a review, see (Owen, McMillan, Laird, & Bullmore, 2005)). A visual version of the task, consisting of two conditions: two-back and zero-back (see Figure 1) was employed. During both of these conditions, 16 letters were singly projected to the center of a screen for 240ms, with an interstimulus interval of 1760ms. The stimuli were white and presented on black background. Each condition was presented for four trials in pseudorandom order and was preceded by a three-second instruction screen (e.g. "Press for 2-back" and "Press for X"). Each trial was followed by a 15-second rest epoch, during which the word 'rest' was projected on to the screen. The entire task lasted 7 minutes and 18 seconds.

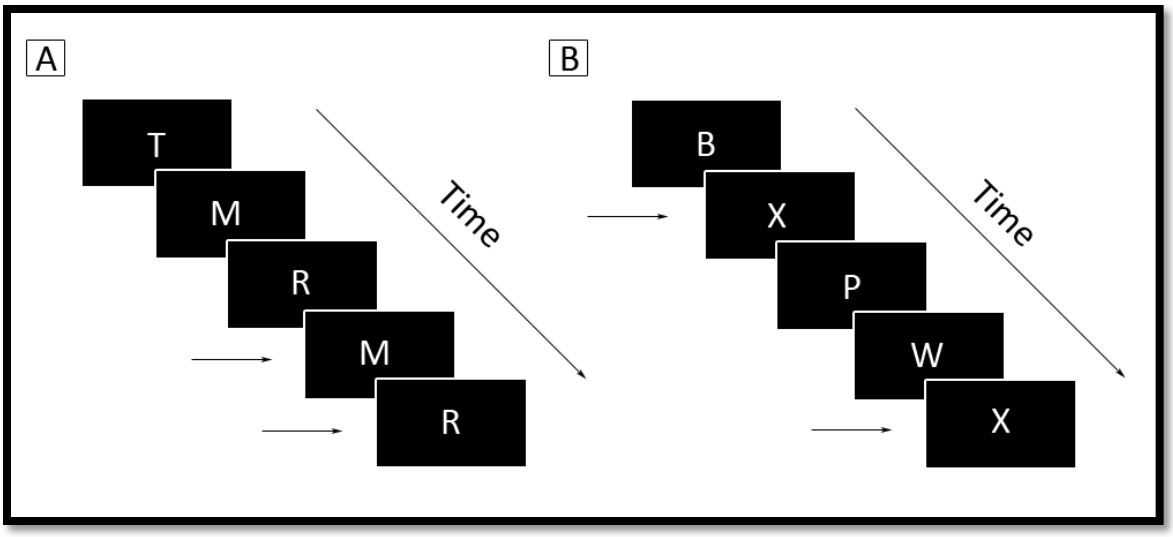

Figure 1. N-back task performed during MRI scanning. A) During the 2-back condition, participants were to respond with a button press only when they observed a letter that was presented two letters ago. B) During the 0-back condition, participants were instructed to

respond with a button press only when the letter ‘x’ appeared on screen. Horizontal arrows indicate correct responses.

Neuropsychological assessment of working memory

Working memory was measured with the digit span and letter-number-sequencing subtests of the Wechsler Adult Intelligence Scale-III (Wechsler 1997), Paced Auditory Serial Addition Test (Fischer, Jak, Kniker, Rudick, & Cutter, 2001; Rao, Leo, Bernardin, & Unverzagt, 1991), Auditory Consonant Trigrams Test (Brown, 1958), Controlled Oral Word Association Test (Delis, Kaplan, & Kramer, 2001), and CNS-Vital Signs Flexibility and Working Memory indices (Gualtieri & Johnson, 2006, 2008). Raw scores from the traditional neuropsychological tests and the index scores from the computerized cognitive tests were combined into a composite summary score. This was done in order to limit the number of comparisons and, consequently, reduce the risk of Type I errors. Raw test scores for each participant, on each cognitive measure, and at each time point were standardized to the means and standard deviations of the corresponding variables in the control group. Further elaboration on the construction of the composite score is provided elsewhere (Collins et al., 2013).

Statistical Analyses

fMRI Task Activation Analyses. Statistical Parametric Mapping 8 (SPM8; www.fil.ion.ucl.ac.uk/spm/software/spm8/) was used to preprocess the imaging data. The functional images were realigned using least-squares minimization to correct for head motion, coregistered and normalized using the segmented anatomical volume, and, finally, smoothed using an isotropic Gaussian spatial filter (FWHM = 6 mm) to reduce noise effects. Where needed, artifacts were detected and repaired using Art Detection Tools (http://web.mit.edu/swg/software.htm). Statistical analyses of the fMRI data were conducted

using the SPM8 implementation of the general linear model (GLM). Contrast images comparing working memory over the control condition (e.g. two-back > zero-back) were created for each participant and used in random-effect analyses at the second-level. Between-group differences in brain activation were calculated using an independent-samples t-test at Times 1 to 3 using a height threshold of $p < .05$ and extent threshold of 50 voxels, corrected for multiple comparisons (e.g. false discovery rate). In order to maximize our statistical power, between-group t-tests were conducted at each time point with the maximum available number of patients, which reduced over time due to attrition. This approach was retained for the functional connectivity analyses.

Functional Connectivity Analyses. The CONN toolbox (Whitfield-Gabrieli & Nieto-Castanon, 2012) for SPM8 was used to conduct seed-to-voxel functional connectivity analyses. Preprocessing of the imaging data progressed the same way as for the activation analyses; however, additional steps were taken to reduce blood oxygen level-dependent contrast signal noise sources that can lead to an increased risk of Type I errors in functional connectivity analyses. Specifically, images were band-pass filtered to 0.01Hz~0.9Hz. Next, following the implemented anatomical component-based noise correction (Behzadi, Restom, Liau, & Liu, 2007), participants' images were regressed on six motion parameters, white matter, cerebrospinal fluid, physiological noise sources (e.g. cardiac and respiratory effects), and task-effects and their temporal derivatives. A 12mm spherical seed located in the dorsolateral prefrontal cortex (dlPFC; MNI: -38, 30, 18) was used to examine FPN-related functional connectivity during working memory processes. This region of the FPN has been previously associated with working memory but not cognitive control (Harding et al., 2014). Within-subject voxel-wise bivariate correlations were calculated between the time courses of the seed region and the whole-brain. Resultant correlation maps were then subjected to Fisher Z-transformations. Between-group

comparisons (e.g. 2nd level GLM) were made at each time point using independent samples t-tests, with a height threshold set at $p < .01$ and extent threshold of 50 voxels, corrected for multiple comparisons.

Demographic Variables, Outcome Measures, and fMRI Task Performance. Demographic and clinical characteristics were analyzed in SPSS 21.0 with t-tests and Chi-square, where appropriate. Neuropsychological data and fMRI task performance data were assessed using mixed design ANOVA.

Results

Baseline demographic and clinical characteristics are presented in Table 1. The groups did not differ in age, education, menopausal status, or inter-scan intervals ($p > .05$).

fMRI Analysis

Patients and controls did not display significant working memory activation differences at Times 1 and 3; however, patients had significantly greater activations at Time 2 in distributed regions (see Table 2). Significant between-group differences of activation were found in the right postcentral gyrus (Brodmann Area [BA] 43) extending to the right temporal lobe, the left occipital pole (BA 17), the left parietal operculum (BA 40) extending to the left temporal lobe and postcentral gyrus, and the left anterior cingulate gyrus (BA 32) extending to left prefrontal regions (Figure 2).

Table 2

Regions of Significantly Greater Working Memory Related Brain Activation in Patients One Month Post-Chemotherapy Relative to Controls

Variable	BA 43	BA 17	BA 40	BA 32
Whole brain				
p-value (FDR corrected)	.000	.001	.007	.000
Cluster size	6270	4152	2661	4653
T-score	5.35	4.48	4.16	3.56
Peak MNI cluster coordinates	58 -14 14	-6 -98 -8	-50 -22 16	-6 36 18
Full cluster description	Right postcentral gyrus, superior/middle temporal lobe, supramarginal gyrus	Left occipital pole, right cuneus, superior/middle occipital lobe	Left parietal operculum, superior and middle temporal gyri, superior and middle frontal gyri	Left anterior cingulate gyrus, superior and middle frontal gyri, right superior frontal gyrus
Contrast value				
Patients, mean (SD)	0.06 (0.16)	0.05 (0.13)	0.03 (0.18)	0.14 (0.20)
Controls, mean (SD)	-0.17 (0.16)	-0.17 (0.18)	-0.20 (0.18)	-0.09 (0.13)
T value	4.56	4.30	4.12	4.33
p-value	.000	.000	.000	.000
Cohen's d	1.44	1.40	1.28	1.36

Note. n=21 for both patient and control groups. Abbreviations: BA, Brodmann area; FDR, false discovery rate; MNI, Montreal Neurologic Institute.

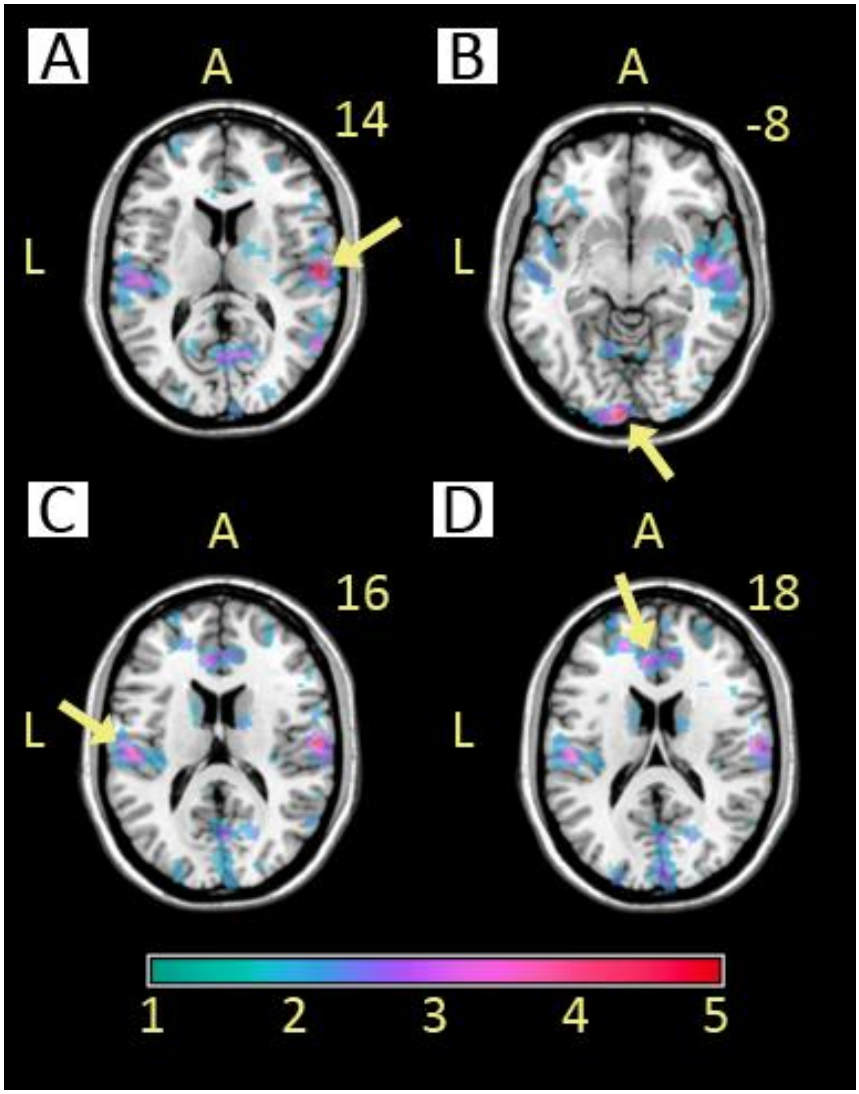

Figure 2. Regions of significantly greater activation for working memory in breast cancer patients compared to healthy controls one month after patient chemotherapy. Patients had increased activity in the (A) right postcentral gyrus (Brodmann area [BA] 43; Montreal Neurological Institute coordinates [MNI]: 58, -14, 14), (B) left occipital pole (BA 17; MNI -6, -98, -8), (C) left parietal operculum (BA 40; MNI -50, -22, 16), and (D) left anterior cingulate gyrus (BA 32; MNI -6, 36, 18). Arrows show peak clusters (height threshold: $p < .05$, extent=20 voxels, corrected for multiple comparisons); the yellow number at the top right of each section displays the axial coordinate position. A indicates anterior and L signifies left. Color bar displays the *T* statistic. Overlays created with MRIcron (http://www.mccauslandcenter.sc.edu/mricro/mricron/).

Functional Connectivity

Comparisons were made between patients and controls to test whether they differed in the degree of functional connectivity displayed in the FPN while performing the two-back condition across Times 1 to 3. Similar to the activation analyses, there were no between group differences at Times 1 and 3. At Time 2, patients demonstrated significantly attenuated functional connectivity between the dlPFC and a cluster in the left occipito-parietal region (see Figure 3).

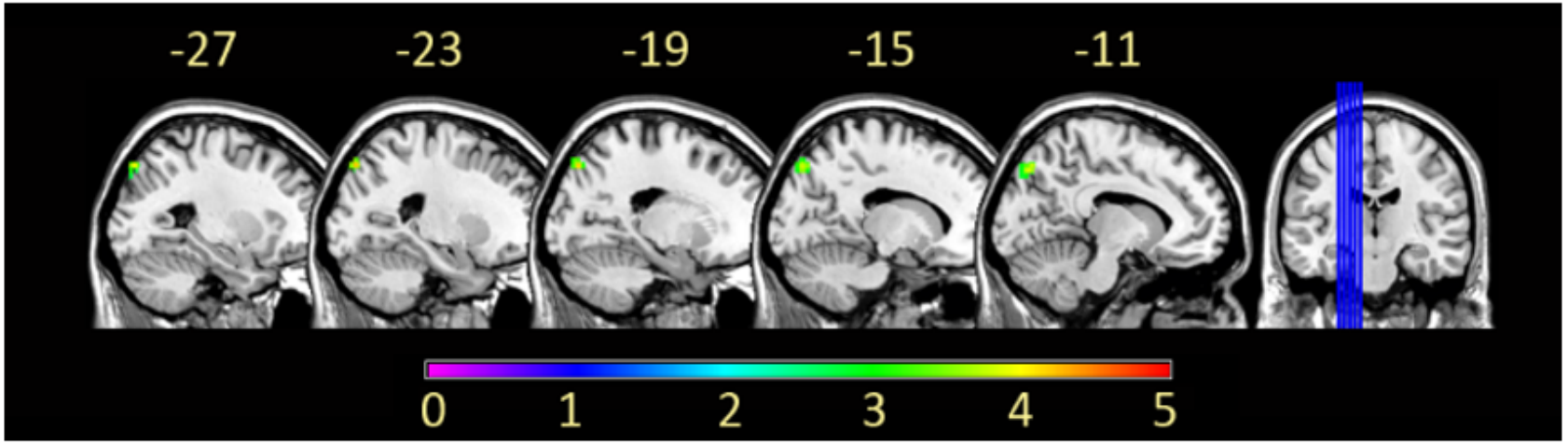

Figure 3. Sagittal maps showing regions of significantly less functional connectivity of the left dorsolateral prefrontal cortex during a working memory task at one month post-chemotherapy in patients as compared to controls. Peak Montreal Neurologic Institute (MNI) coordinates: -22, -82, 50; cluster size: 597 voxels; *p*-value = .001, corrected for multiple comparisons. Color bar shows T score range. Slice labels display sagittal coordinates. Overlays were created with MRIcron (http://www.mccauslandcenter.sc.edu/mricro/mricron/).

Performance on neuropsychological battery and fMRI working memory

The mean scores for the working memory composite and its components are shown in Table 3. There was no significant group-by-time interaction on the composite score, nor were there significant differences between groups at any individual time point.

With respect to n-back task performance, there was a main effect of group membership for reaction time, with patients taking significantly longer to provide responses across all three time points, $F(1,31) = 5.72$, $p < .05$, partial $\eta^2 = .16$; see Figure 4). The groups did not differ in terms of errors made ($p > .05$). There was no group-by-time interaction on either reaction time or errors.

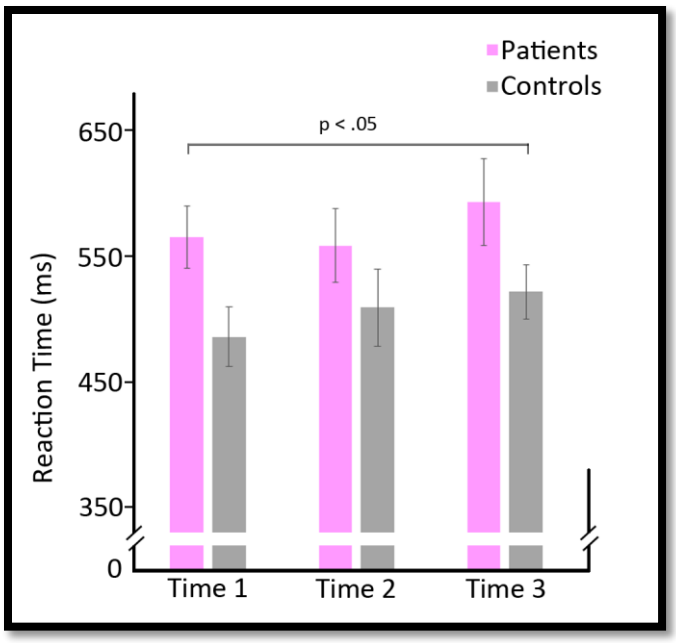

Figure 4. fMRI N-Back task performance results. Patients had significantly greater reaction times than controls across time points. Error bars represent the standard error of the mean.

Table 3

Means (Standard Deviations) of Outcome Measure Data

Measures	**Time 1**		**Time 2**		**Time 3**		*p*-value
	Patients ($n = 19$)	Controls ($n = 19$)	Patients ($n = 19$)	Controls ($n = 19$)	Patients ($n = 19$)	Controls ($n = 19$)	
Working Memory Composite Score	-0.26 (0.71)	0.00 (0.68)	-0.35 (0.72)	0.00 (0.60)	-0.36 (0.70)	0.00 (0.69)	> .05
WAIS-III Digit Span	17.63 (4.00)	16.79 (4.83)	19.68 (4.60)	18.53 (4.31)	18.84 (4.35)	18.21 (4.41)	-
WAIS-III Letter-Number Sequencing	10.26 (2.35)	11.00 (2.96)	11.79 (2.64)	12.16 (2.87)	11.47 (2.72)	12.16 (2.79)	-
PASAT	47.16 (10.71)	51.16 (6.10)	52.16 (9.04)	56.16 (4.51)	52.16 (7.65)	55.11 (6.83)	-
ACT	35.00 (5.58)	37.26 (4.82)	36.32 (4.55)	40.00 (4.32)	35.89 (8.01)	36.63 (10.38)	-
COWAT	39.02 (12.84)	50.63 (16.71)	41.84 (14.41)	50.63 (16.71)	41.74 (13.01)	47.47 (14.37)	-
CNS-VS Flexibility	47.74 (11.80)	61.58 (8.38)	56.58 (9.25)	61.58 (8.38)	55.39 (8.73)	60.95 (7.80)	-
CNS-VS Working Memory	11.00 (3.77)	13.21 (2.10)	12.32 (2.93)	13.21 (2.10)	11.28 (3.77)	12.63 (3.00)	-

Note. Means and standard deviations based on raw scores on traditional neuropsychological tests; Working memory composite scores represent average of Z-scores (referenced to control group mean and standard deviation at same time point) on tests comprising that domain, as described in the Methods section. WAIS: Wechsler Adult Intelligence Scale; PASAT: Paced Auditory Serial Addition Test; ACT: Auditory Consonant Trigrams Test; COWAT: Controlled Oral Word Association Test; CNS-VS: CNS-Vital Signs.

Discussion

This is the first study, to our knowledge, that has prospectively examined functional connectivity in breast cancer patients compared to healthy controls. The findings from this study play an important role in understanding the neural underpinnings of CRCI in this population. Notably, we found that at an average of one month after chemotherapy, breast cancer patients had reduced functional connectivity in the frontoparietal network, and that patients recruited significantly broader brain regions when completing a working memory task. These differences were not observed at baseline or one year post-chemotherapy, strengthening the evidence of the acutely injurious effects of chemotherapy.

In examining the susceptibility of the FPN to chemotherapy, this study makes a unique contribution to the CRCI neuroimaging literature. Although the FPN is broadly implicated in flexibly allocating cognitive resources across various mental demands, the FPN is also critical for working memory (Harding et al., 2014). In the present study at Time 2, patients had reduced functional connectivity between a region of the FPN in the left dlPFC and the left occipito-parietal region. Each of these regions has been shown to support differing functions of working memory. The left dlPFC has been implicated in the manipulation of verbal information (Barbey, Koenigs, & Grafman, 2013), whereas the occipito-parietal cortex has been associated with the storage of verbal information during working memory tasks (Jonides et al., 1998). In addition, previous work has found evidence of reduced white matter integrity in the left parietal region of the superior longitudinal fasciculus (SLF) in breast cancer patients three to five months post-chemotherapy (Deprez et al., 2012). The SLF is a white matter tract that supports communication between the occipito-parietal and frontal regions (Schmahmann & Pandya, 2006), and it has been related to working memory in healthy and pathological populations (Walsh et al., 2011). Taken

together, our finding, along with previous work, offers accumulating evidence of working memory-related brain network disruption shortly after chemotherapy.

The pathological mechanism of injury to this network remains to be fully understood. Although there is controversy surrounding the sensitivity of diffusion tensor imaging to detect white matter demyelination (Wheeler-Kingshott & Cercignani, 2009), others have proposed that this injurious process and axonal injury may be related to changes in the microstructure of white matter in CRCI (de Ruiter et al., 2012; Deprez et al., 2012). Previous work has shown reductions of myelin and oligodendrocyte precursors subsequent to the administration of 5-fluorouracil in mice (Weng et al., 2014). Transiently reduced functional connectivity between areas connected by the SLF may be a consequence of white matter demyelination of the tracts that support the working memory component of the FPN. Subsequent recovery of functional connectivity in this network may be attributable to remyelination, which has been reported in clinical populations (for a review, see Franklin & ffrench-Constant, 2008; Barkhof et al., 2003).

Interestingly, although we found reduced functional connectivity within working memory-related circuitry of the FPN, patients and controls did not differ in terms of correct and incorrect responses made during the fMRI task. The groups differed in terms of reaction time; however, this finding appears unrelated to chemotherapy, as it was significant across time, including at pre-treatment. This is consistent with prior work that reported similar baseline reaction time differences (Ahles et al., 2008).

Imaging results at Time 2 revealed expansive brain hyperactivations for the breast cancer patients, with the most significant increases in neural activity occurring in the right postcentral gyrus and left anterior cingulate gyrus regions. The anterior cingulate has long been recognized for its role in action monitoring (Carter et al., 1998; MacDonald, Cohen, Stenger, & Carter,

2000), thus its increased activation during a working memory task at Time 2 in the patient group may reflect increased attention to performance. In light of the similar performance between groups during the working memory task (e.g. errors during the two-back condition), these imaging findings support a hypothesis advanced by our group and others that breast cancer patients may be engaging in neural compensation in order to perform at, or near, premorbid levels (McDonald, Conroy, Ahles, et al., 2012; Scherling & Smith, 2013).

Although working memory composite scores from the neuropsychological battery were not statistically different between breast cancer patients and controls, it is important to highlight that patients did, on average, perform more poorly than controls cross-sectionally at each time point. An absence of statistical significance raises the possibility that CRCI does not directly impact working memory; however, others have reported significant differences between breast cancer patients and controls in this domain one month after chemotherapy (Stewart et al. 2008), and we observed a significant decline in working memory when analyzing data from the full sample of breast cancer patients from which this subsample was drawn (Collins et al., 2014). This suggests that failure to achieve statistical significance in the current study is due to a lack of power resulting from our limited sample size. Considering our findings of broad neural hyperactivations and disrupted functional connectivity shortly after treatment in the present sample, these findings suggest that neuroimaging may be more sensitive than neuropsychological measures to the subtle brain effects of peripheral chemotherapy administration. Interestingly, Collins et al. (2014) reported that working memory significantly improved one year post-chemotherapy, in line with our study's finding of functional recovery at Time 3. Additionally, we have previously reported that one-year post-chemotherapy, breast cancer patients from this cohort displayed grey matter recovery in regions associated with working memory circuitry

(Lepage et al., 2014). Although cross-sectional CRCI studies have reported brain irregularities and cognitive dysfunction in executive functions, processing speed, and memory many years after chemotherapy (Ahles et al., 2002; de Ruiter et al., 2011, 2012; Koppelmans et al., 2011, 2012; Silverman et al., 2007; Yamada, Denburg, Beglinger, & Schultz, 2010), there is a paucity of evidence of chronic working memory dysfunction. Taken together, these findings suggest that working memory is acutely vulnerable to the cognitively injurious effects of chemotherapy, but may not be a component of long-term CRCI.

A theme that is emerging from longitudinal neuroimaging studies of CRCI is one of structural and functional brain abnormalities during and shortly after treatment, with some degree of recovery in the year following chemotherapy cessation (Dumas et al., 2013; Lepage et al., 2014; McDonald et al., 2010; McDonald, Conroy, Ahles, et al., 2012). To our knowledge, this study is the first to report a complete return to baseline by one year post-treatment. A prior longitudinal study of working memory in the breast cancer population found that despite some recovery one year after chemotherapy, frontal hyperactivations and parietal hypoactivations were still present during an auditory n-back task (McDonald, Conroy, Ahles, et al., 2012). The variations in our findings may be attributable to the differing study characteristics, including the presentation of stimuli (e.g. visual compared to auditory presentation), which has been shown to elicit different frontoparietal activations in healthy controls (Crottaz-Herbette, Anagnoson, & Menon, 2004). Similarly, we did not find any pre-treatment working memory-related functional differences, in contrast to previous work by others and by our group (Cimprich et al., 2010; López Zunini et al., 2013; McDonald, Conroy, Ahles, et al., 2012; Scherling et al., 2011). These studies differed in terms of included chemotherapy regimens, working memory tasks employed, and different comparative groups, such as healthy and chemotherapy-free breast cancer controls,

which may contribute to the variability in the findings across studies. Furthermore, differential findings among studies may also be influenced by patients who are particularly vulnerable to chemotherapy, even before the commencement of treatment. A recent prospective study has found that pre-treatment brain functional inefficiency, signalled by broad spatial variance within frontoparietal regions during working memory, significantly predicted cognitive complaints in breast cancer patients (Askren et al., 2014). Previous work has also demonstrated a relationship between reduced processing speed performance and lower cognitive reserve in chemotherapy-treated breast cancer patients (Ahles et al., 2010); however, cognitive reserve and its influence on CRCI remains an understudied topic. In light of work showing that some women experience cognitive dysfunction prior to chemotherapy, future work should look to pre-treatment cognitive reserve as a possible modulating factor of later CRCI.

Limitations of this study include the lack of a non-chemotherapy breast cancer control group. While others have included such a control group in a prospective study and obtained similar functional results (McDonald, Conroy, Ahles, et al., 2012), we could not control for cancer-related factors. Although our participants were closely matched at baseline, all patients became menopausal by Time 3, whereas controls remained unchanged with respect to menopausal status. Subsequent to chemotherapy, treatment-induced menopausal symptoms have been reported to co-occur alongside cognitive disruption (Fan et al., 2005); thus, future longitudinal studies should account for menopausal status. The types of surgery, number of cycles and type of chemotherapy varied among the patients (see Table 1). From Time 2 to Time 3, some patients received radiotherapy or commenced hormonal therapy. Although these adjuvant treatments have been associated with cognitive decline (Bender, Paraska, Sereika, Ryan, & Berga, 2001; Quesnel et al., 2009), the sample size of the present study precluded their

investigation. In order to overcome the shortcomings that are common between this and other neuroimaging studies of CRCI, it will be important for future research to strive to be multicenter and collaborative.

In summary, the current study offers the first prospective report of frontoparietal network functional connectivity disruption in chemotherapy-treated breast cancer patients. Also unique to this study is evidence of a neurofunctional return to baseline in working memory-related circuitry around one year post-chemotherapy. Although there are still many questions to answer about CRCI, this study provides hope for patients who experience trouble with working memory both during and following chemotherapy. As women are being more informed about the potential for CRCI as they begin treatment, this article provides evidence for recovery of the neural underpinnings of working memory and, thus, may reduce the fear of permanent consequences of treatment.

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General Discussion

The overarching goal of this thesis was to use contemporary neuroimaging techniques to better characterize the chemotherapy-related neurophysiology underlying the cognitive disruption experienced by chemotherapy-treated breast cancer patients. By prospectively investigating the relationship of both brain structure and function to cognitive functioning in chemotherapy-exposed breast cancer patients, this thesis provides novel insights about the extent of CRCI. To achieve its goal, the thesis comprised two correlational studies in which brain structure and function were contrasted between breast cancer patients and cancer-free controls before patients began chemotherapy, as they completed it, and one-year after treatment.

In the following sections, both studies of the thesis are recapitulated, with special attention drawn to their principal findings. The limitations of the works are then discussed and blended with suggestions for future research. The final section offers an overall summary and concludes the thesis.

Grey matter volumes and overall cognitive function

The first study (Study 1) had a primary aim of investigating the relationship between grey matter alterations and cognitive function. Although previous work established a link between grey matter fluctuations and cognitive compromise in chemotherapy-treated breast cancer patients (Conroy et al., 2012; McDonald, Conroy, Ahles, West, & Saykin, 2010; McDonald, Conroy, Smith, West, & Saykin, 2012), a prospective study incorporating a robust neuropsychological battery was lacking in the literature. Thus, Study 1 served the important purpose of characterizing, not only the areas of the brain that may be negatively impacted over time by peripheral chemotherapy, but also how these areas relate to cognitive function.

With respect to the course of grey matter attenuation, the hypothesis that greater disruption would be greatest shortly after chemotherapy, and partially resolve one year later, was supported. Given the paucity of longitudinal grey matter studies in the CRCI literature, analyses comprised whole-brain volumes rather than *a priori* regions-of-interest. Approximately one month after chemotherapy completion, breast cancer patients had grey matter volume loss in widespread regions. Medial and inferior frontal regions, along with temporal areas were the greatest to be acutely impacted, in line with previous studies (Inagaki et al., 2007; McDonald et al., 2010; McDonald et al., 2012). Similar to prior work (de Ruiter et al., 2012) showing parietal volume reductions, the paracentral lobule and the precuneus within the right hemisphere were also disrupted shortly after chemotherapy. The functional neuroimaging literature of CRCI highlights abnormal task-related activations primarily in frontal and parietal regions (for a review, see (Pomykala, de Ruiter, Deprez, McDonald, & Silverman, 2013)). Since those regions are commonly recruited during cognitive processes frequently explored in CRCI (e.g. working memory), it remained unclear whether the circumscribed nature of the irregularities reflected task demands, or whether frontoparietal regions might instead be more sensitive to chemotherapy than other areas. In absence of frontoparietal-driven demands, Study 1 lends support for the latter, adding to the evidence from other structural studies showing increased chemotherapy susceptibility in these regions (de Ruiter et al., 2012; Inagaki et al., 2007; McDonald et al., 2012).

Cognitive function was assessed using a comprehensive neuropsychological battery and was categorized as follows: information processing speed, working memory, verbal memory, and visual memory. Although the larger sample from which participants of Study 1 were drawn displayed significantly decreased working memory over the course of treatment (Collins,

MacKenzie, Tasca, Scherling, & Smith, 2013), information processing speed was the only domain to reach significance in the Study 1 participants. Mean scores on the three other domains tended to decrease, but the differences were not significant. Information processing speed displayed the greatest relationship with a range of disrupted areas, particularly in prefrontal, temporal, and superior parietal areas. Although less robust, visual memory was related to areas in the prefrontal cortex, and working memory was positively associated with bilateral frontal grey matter. Verbal memory did not show an association with regions of reduced grey matter.

Acute grey matter volume losses tended to resolve by one-year post-chemotherapy. However, continued disruption was noted largely within inferior and medial frontal regions, the anterior cingulate, the middle frontal gyrus, and in medial temporal areas. Medial frontal regions have been previously shown to have persistent reductions of grey matter volume following chemotherapy (McDonald et al., 2010). Study 1 found working memory was positively associated with the right middle frontal gyrus, a region demonstrating sustained hypoactivity during a working memory task in breast cancer survivors one year after treatment (McDonald et al., 2012). Taken together, the findings from Study 1 suggest that a potential predilection for the prefrontal cortex by chemotherapy may underlie the working memory deficits experienced by breast cancer patients.

Working memory and the frontoparietal network

The aim of the second study (Study 2) was to elucidate the neural circuitry of the working memory impingement associated with chemotherapy. As the most acutely vulnerable cognitive domain in the context of CRCI (Stewart, Bielajew, Collins, Parkinson, & Tomiak, 2006), working memory has been the subject of increasing investigation using functional neuroimaging; however, prospective studies remain scarce. Study 2 sought to replicate previous studies of

working memory task-related activity over the course of treatment, and to incorporate cutting edge analytical methods to explore a functional network within commonly disrupted brain regions.

Prior to treatment, breast cancer patients and controls did not display any functional differences, contrary to prior reports of baseline working memory related frontal, insular, and diencephalic hyperactivations, and parietal hypoactivations (McDonald et al., 2012; Scherling, Collins, Mackenzie, Bielajew, & Smith, 2011). By one month after treatment, breast cancer patients had expansive working memory associated hyperactivations in frontal, temporal, and parietal regions when compared to controls. Remarkably, these hyperactivations resolved by one-year post-treatment. Although a previous longitudinal study reported a partial return to baseline working memory activations at the one-year post-chemotherapy interval (McDonald et al., 2012), two important distinctions should be noted. First, McDonald et al. (2012) reported that breast cancer patients had baseline working memory *hyperactivations* in frontal and parietal regions, and *decreased* inferior frontal activity by one-month post-treatment. Thus, the directionality of activity between Study 2 and that study is opposite. Next, Study 2 is the first to report a complete resolution of abnormal functional activity by the one-year interval. This finding is supported by recovery of working memory in the larger sample of chemotherapy-treated breast cancer patients from which the Study 2 cohort was drawn (Collins, Mackenzie, Tasca, Scherling, & Smith, 2014).

The frontoparietal network (FPN) was explored using functional connectivity - an analysis method to correlate neural activity between regions across a timeseries (Friston & Buchel, 2003). Thus, this technique allows for inference based on the co-activation of regions, which can suggest brain network function. Beginning with a region in the left dorsolateral

prefrontal cortex (dlPFC), the timeseries across the entire brain was explored to determine areas significantly activated alongside this region. Given the selected region of the dlPFC was chosen for its role in working memory (Harding, Yücel, Harrison, Pantelis, & Breakspear, 2014), it was not surprising to find that it was functionally correlated in time to an area in the left superior parieto-occipital region. However, it was noteworthy that this connection was significantly disrupted in breast cancer patients one-month post-treatment, but not at baseline and one-year follow-up. Previous work has illustrated a similar pattern within the dorsal attention network, with disruption between frontoparietal regions (Dumas et al., 2013). However, in that study, an identical analysis of the default mode network, disruption between the posterior cingulate cortex and precuneus persisted to the final assessment interval. Thus, Study 2 adds to the accumulating evidence that, despite a pronounced, acute disruption in working memory substrates, regions supporting this cognitive function recover over time, in line with neuropsychological performance in this domain (Collins et al., 2014).

Overall, this thesis adds to the growing body of CRCI neuroimaging literature that identifies the short-term post-treatment period to be the most susceptible to neural and cognitive disruption. Study 1 makes a novel contribution to the field of CRCI research by exploring the relationship of grey matter attenuation and a number of objectively assessed cognitive domains. Study 2 is the first prospective functional connectivity study conducted in the CRCI literature. It is also the first to demonstrate a recovery to pre-treatment neural activity, despite short-term working memory circuitry abnormalities. An overarching strength of both studies was the inclusion of a comparison group that was matched to the patient group on age and education, thus controlling for potential confounding by these variables.

Limitations and future directions

Although the studies of this thesis contribute novel findings to the CRCI literature, some limitations herein warrant acknowledgement. Since both studies were conducted on the same sample, and neuroimaging data were collected during the same sessions for each study, most of the limitations apply equally to both investigations. Although patients and controls were closely aligned on important characteristics, such as age, and education, they were not matched on disease and treatment. The control group comprised cancer- and chemotherapy-free women. With the absence of a disease-matched group, the influence of potentially cancer-related processes on cognition could not be controlled. Despite this limitation, the findings of both studies are congruent with previous studies of grey matter volume (McDonald et al., 2010), and working memory fMRI investigations that included chemotherapy-free breast cancer controls (McDonald, Conroy, Ahles, et al., 2012).

Within the patient group, there was variability in terms of the number of cycles, and types of chemotherapy. Teasing apart differential contributions to CRCI by various chemotherapy agents remains a challenge in human studies. Animal studies indicate that the magnitude of neurotoxicity is contingent on the chemotherapy agent administered, in addition to other variables, including animal model, and type of test used (for a review, see (Seigers & Fardell, 2011)). In the context of human studies of CRCI, some neuroimaging and neuropsychological studies have explored the effects of single regimes (e.g. (Collins et al., 2013; Koppelmans, Breteler, et al., 2012; Koppelmans, de Ruiter, et al., 2012)); however, comparing the effects of different agents remains a challenge.

Similarly, some patients received radiotherapy or hormonal therapy as treatment progressed. Although these adjuvant treatments have been associated with cognitive decline (Bender, Paraska, Sereika, Ryan, & Berga, 2001; Collins, Mackenzie, Stewart, Bielajew, &

Verma, 2009; Quesnel, Savard, & Ivers, 2009), sample size limitations prevented their exploration in this thesis. Interestingly, despite the potential confounding effects of these treatments, patients experienced a recovery of grey matter, and working memory related neural activity patterns. In addition, although estrogen and antiestrogen treatments appear to negatively influence verbal memory in particular (Collins et al., 2009; Ryan, Scali, Carriere, Ritchie, & Ancelin, 2008), this ability was not related to regions of grey matter loss reported in Study 1. In all, these findings suggest the potential effects of hormonal and radiation therapy may have been minimal, if they existed.

Participants were closely matched prior to patient treatment; however, patients became menopausal over the course of chemotherapy, whereas controls remained unchanged in this regard. Treatment-induced menopausal symptoms are associated with cognitive compromise following chemotherapy in breast cancer patients (Conroy, McDonald, Ahles, West, & Saykin, 2013; Fan et al., 2005), warranting further research to elucidate this confounding variable.

Both Study 1 and 2 are part of a larger research project from which several studies have emerged. A striking difference between Study 2 and previously published fMRI studies conducted by our research group is the absence of pre-treatment activation differences. A pre-treatment visual working memory study by our group revealed frontal, insular, thalamic, and midbrain neural hyperactivations in breast cancer patients (Scherling et al., 2011). Similarly, pre-chemotherapy response inhibition in the same sample revealed abnormal frontal and cerebellar activations (Scherling, Collins, Mackenzie, Bielajew, & Smith, 2012). Verbal recognition associated pre-treatment insular, orbitofrontal, middle temporal, and anterior cingulate hyperactivations have also been found in our sample (López Zunini et al., 2013). These studies support work from other groups that have demonstrated pre-chemotherapy neuropsychological

performance (Wefel, Lenzi, Theriault, Davis, & Meyers, 2004) and brain function (Cimprich et al., 2010) disruptions in breast cancer patients. The work from our group has demonstrated that variables such as estrogen, cortisol, metacognition, days since surgery, depression, anxiety, task errors (commission and omission), reaction time, and analytic approach may impact activation profiles. A lack of baseline group differences in Study 2 may reflect the omission of covariates during analysis, and subtle differences in cognitive demands between fMRI tasks. The use of covariates in Studies 1 and 2 was restricted in an attempt to protect from Type I errors. To tease apart the influence of the many factors that have been suggested to moderate CRCI-related brain activity, multisite collaborations are needed to pool together larger samples.

A number of risk factors and postulated mechanisms of impairment were outlined in the general discussion, but were not examined in this thesis. These offer ripe opportunities for future research, especially when combined with contemporary neuroimaging techniques. One of the most salient lines of inquiry in CRCI pertains to the mystery of how chemotherapy exerts neurotoxic effects. The blood-brain barrier (BBB) is remarkable in its ability to prevent substances from coming into contact with brain parenchyma and continues to stifle the use of cytotoxic agents in the treatment of brain tumours (Deeken & Löscher, 2007). In spite of evidence from animal studies that certain commonly used agents, such as 5-fluorouracil, can penetrate the rodent BBB (Sakane, Yamashita, Yata, & Sezaki, 1999), evidence in humans is lacking.

Recent work using dynamic contrast-enhanced MRI in the field of Alzheimer's research suggests that age-related breakdown of the BBB can occur in the hippocampus (Montagne et al., 2015). Although Montagne et al. observed more pronounced BBB breakdown in MCI patients in contrast with neurologically healthy controls, their findings have important implications for

CRCI research. The use of dynamic contrast-enhanced MRI is relatively nascent in MRI research (Armitage, Farrall, Carpenter, Doubal, & Wardlaw, 2011; Heye, Culling, Valdés Hernández, Thrippleton, & Wardlaw, 2014); however, the use of this technology in CRCI may elucidate how neurotoxicity via peripheral chemotherapy administration occurs. Next, age-related reductions in the protection provided by the BBB are portentous for a disease that increases in incidence as women age. Future work should investigate whether age-associated weakening of the BBB increases the risk of CRCI in breast cancer patients as they age. Finally, the hippocampus is disrupted in function and size in human and animal studies of CRCI (Bergouignan et al., 2011; Christie et al., 2012; de Ruiter et al., 2011; Kesler, Janelsins, Koovakkattu, & Palesh, 2013; Seigers et al., 2009). Study 1 of this thesis found the right hippocampus had persistent volume loss one-year post-treatment and was marginally significantly associated with processing speed. Others have linked chemotherapy-related hippocampal volume loss to memory retrieval deficits (de Ruiter et al., 2011; Kesler et al., 2013). Future studies should investigate the role of age-related hippocampal BBB disruption and CRCI, particularly in light of studies showing intact hippocampi post-treatment (Koppelmans et al., 2012; Yoshikawa et al., 2005). These mixed findings in the CRCI literature may be associated to age-related BBB disruption.

Cancer patients have increased levels of cytokines compared to healthy controls, with evidence that chemotherapy induces an even greater presence of these proteins (Vardy et al., 2007). Among other functions, cytokines instigate inflammation, and oxidative stress in the brains of animal models (Joshi et al., 2010; Tangpong et al., 2007). In breast cancer patients, alterations of cytokine levels (e.g. interleukin-6, tumour necrosis factor-alpha) have been associated with hippocampal size and verbal memory performance (Kesler et al., 2013). As well, increased levels of pro-inflammatory cytokines have been associated with cognitive complaints

and abnormal brain metabolism (Ganz et al., 2013; Pomykala, Ganz, et al., 2013). There is evidence that chemotherapeutic agents frequently used to treat breast cancer modulate cytokine responses differently (Janelsins et al., 2012) and that increases in some, coupled with decreases in others, are associated with reduced volume in grey matter structures (Kesler et al., 2013). Thus, although this thesis did not examine the effects of cytokines, they are increasingly being recognized as a potential candidate mechanism for CRCI, and they should be further examined in future studies.

Genetic vulnerability and cognitive reserve have been identified as potential risk factors for CRCI; however, their contributions to CRCI are only beginning to be investigated (Ahles et al., 2003, 2014; Ferguson, McDonald, Saykin, & Ahles, 2007). Although these factors were not explored in this thesis, their potential influence should be studied in the future.

As the list of potential confounds that were presented in this thesis suggests, CRCI is a complex, multifaceted phenomenon. With respect to neuroimaging investigations, sample sizes often limit researchers' ability to explore the role of numerous variables due to the inherently expensive nature of such studies. In the CRCI literature, sample sizes can be further limited by the challenge of recruiting patients experiencing a life-threatening illness and its disruptive treatments. With an aim to better characterize CRCI, related neuroimaging studies would benefit from multi-centre collaborations. This would allow researchers to pool together imaging data in order to have adequate statistical power to tease apart the roles of confounds that have yet to be fully understood.

As this thesis illustrates, neuroimaging is an effective tool that can describe brain morphology and function related to cognitive decline. MRI is also a useful tool to explore relationships between variables of interest, such as cytokine levels, age, etc., and brain related

activity and structure, both statically and over time. In the context of increasing our understanding of CRCI, a much-anticipated next step for MRI technology would be to predict who might be expected to experience cognitive decline and recovery. In psychiatric populations, functional neuroimaging has been used to improve the prediction of treatment response using pre-treatment task-related neural signatures (Doehrmann et al., 2013) and metabolic activity (Fan, Resnick, Wu, & Davatzikos, 2008). These approaches may be well suited to extend the application of research paradigms into clinical practice, increasing evidence-based treatment considerations for cancer patients. Thus far, emerging analytical approaches to MRI data have shown promising results in terms of differentiating chemotherapy-treated from chemotherapy-free patients and healthy controls (Kesler et al., 2013). The predictive potential of MRI is an exciting and nascent area in CRCI research that deserves increased attention.

With the accrual of CRCI evidence, research should also look beyond description and prediction of CRCI and into remediation of treatment-related cognitive dysfunction. Increased prediction of those who will be at risk of CRCI will be helpful to make treatment-related decisions; however, chemotherapy is likely to continue to be used as an adjuvant treatment for the foreseeable future. Thus, current issues facing cancer patients must be addressed. A limited number of studies have endeavoured to explore the impact of cognition-oriented psychological interventions in breast cancer survivors (for a review, see (Sleight, 2015)). Although the results are mixed, promise has been found in terms of improved executive function (e.g. cognitive flexibility, verbal fluency, and processing speed) (Kesler et al., 2013), and verbal memory (McDougall, Becker, Acee, Vaughan, & Delville, 2011).

Incorporating MRI-based interventions into existing treatment approaches may offer an opportunity to improve outcomes in chemotherapy-treated cancer patients with cognitive

dysfunction. Neurofeedback using real-time fMRI allows participants to exert cognitive control over regional blood oxygen level-dependent signals, with the aim of altering outcomes and behaviour (for a comprehensive review, see (Sulzer et al., 2013)). Of interest, there is preliminary evidence that the dlPFC can be self-regulated with the assistance of real-time fMRI feedback (Zhang, Yao, Zhang, Long, & Zhao, 2013). Notably, Zhang et al. reported that, in addition to showing increased activity in the left dlPFC, the experimental group made significant improvements on working memory measures. Conversely, the control group was unchanged in focal brain activity or working memory performance. In light of findings of Study 2, real-time fMRI provides an interesting opportunity to mediate short-term working memory neural dysfunction in breast cancer patients.

Conclusion

Chemotherapy remains the primary post-surgical intervention for breast cancer patients. Both the prevalence of breast cancer, and the associated negative side-effects related to its treatment compel research institutions and their scientists to uncover ways to improve the quality of life for the millions of impacted women worldwide. An area that has received increasing attention is the influence of chemotherapy on cognition. With an impairing effect on cognitive abilities such as working memory, executive function, processing speed, and learning and memory, chemotherapy is a Trojan horse treatment.

The strength of evidence for its damaging reach on cognition has accumulated, with longitudinal, prospective studies clarifying the course and related factors of chemotherapy-associated cognitive decline. Neuroimaging studies have contributed to the characterization of CRCI, but longitudinal studies of this nature are few. This thesis aimed to provide further

clarification on the structural and functional consequences of chemotherapy, and did so with two prospective investigations.

As has been emerging from the CRCI literature, this thesis found that the injurious effects of chemotherapy are most evident shortly after treatment completion. Novel findings were made in each included study. The first study discovered an association between broad regions of grey matter volume loss with neuropsychological functioning across a number of domains. The second study provided evidence of working memory related brain disruption that existed only at the short-term post-treatment interval. A noteworthy return to baseline activity was noted by one-year after treatment, an as-yet unseen finding in the CRCI literature.

In summary, the studies of this thesis contribute to the growing body of literature that describes the course of disruption in the neural substrates of the cognitive abilities that are compromised following adjuvant chemotherapy. Despite an observed longer-term recovery, the short-term brain disruption experienced by breast cancer patients warrants increased attention. Neuroimaging research is well poised to make improvements in the care of breast cancer patients. It is hoped that future research will use this technology to inform treatment decisions and be used to alleviate chemotherapy-related cognitive impairment.

This research can aid patients and their doctors become better informed about the potential cognitive sequelae of chemotherapy. Women slated to undergo chemotherapy should be made aware that they might experience cognitive difficulties associated with treatment. Moreover, for women who experience working memory related difficulties during and after chemotherapy, it is hoped that this research will offer a degree of optimism for a potential resolution of the treatment-related cognitive dysfunction.

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