What happens before chemotherapy?!
Neuro-anatomical and -functional MRI investigations of the pre-chemotherapy breast cancer brain.

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General Abstract

The side-effects of chemotherapy treatment are an increasingly important research focus as more cancer patients are reaching survivorship. While treatment allows for survival, it can also lead to problems which can significantly affect quality of life. Cognitive impairments after chemotherapy treatment are one such factor. First presented as anecdotal patient reports, over the last decade empirical evidence for this cognitive concern has been obtained.

Much attention has been focused on post-chemotherapy research, yet little attention has been granted to these same patients' cognition before treatment commences. Breast cancer (BC) patients face many obstacles before chemotherapy treatment such as: surgery and side-effects of anesthesia, increased cytokine activity, stress of a new disease diagnosis and upcoming challenges, and emotional burdens such as depression and anxiety. Many of these factors have independently been shown to affect cognitive abilities in both healthy populations as well as other patient groups. Therefore, the pre-treatment (or baseline) BC patient status warrants systematic study. This would then reduce mistakenly attributing carried-over cognitive deficits to side effects of chemotherapy. As well, it is possible that certain confounding variables may have neural manifestations at baseline that could be exacerbated by chemotherapy agents.

The following thesis first presents a review paper which critically describes the current literature examining chemotherapy-related cognitive impairments (CRCIs), as well as possible confound variables affecting this population. Subsequently, three original research papers present pre-chemotherapy data showing significant neuroanatomical and neurofunctional differences in BC patients compared to controls. In particular, these neural differences are present in brain regions that have been reported in post-chemotherapy papers. This, as well as the effects of variables such as the number of days since surgery, depression and anxiety scores and more, support the initiative that research attention should increase focus on these patients at baseline in order to better understand their post-chemotherapy results.

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Chapter 1

General Introduction

Chemotherapy is a familiar treatment in society today. Even without a personal cancer scare, rare is the person who has escaped knowing someone who has had cancer. On average, 445 Canadian women will be diagnosed with breast cancer every week, amounting to an estimated 23,200 women every year [1]. A cancer diagnosis not only affects the body, but a whole network of factors, such as relationships with loved-ones, professional status and ambitions for the future. Equally, survivorship from cancer can come with repercussions. One side-effect of chemotherapy that has been reported by breast cancer patients in particular, and has gained research support over the last decade, is cognitive impairment. While this change in cognition can be transient in nature for certain patients, it may also persist for years and can seriously affect the quality of life of cancer survivors and those around them. Patients have coined the terms "chemofog" or "chemobrain" to explain what they experience. This implies a direct link between cognitive issues and chemotherapy treatment. While some research has supported this difference between chemotherapy treated patients and controls, particularly in areas of cognition such as memory, the patient's cognitive ability prior to chemotherapy has been neglected in the literature, and the impact of factors such as surgery [2] and cytokine activity [3] on cognitive abilities prior to treatment have been virtually ignored. Given the stress of a cancer diagnosis and the rigorous decision-making that inevitably is required afterwards, intact cognitive abilities are essential. If these cancer patients are cognitively impacted during this time, it is important for the medical professionals, family and friends involved in decision making with the patient to know. Similarly, these cognitive impairments reported by patients after chemotherapy may not be solely due to side-effects of treatment, but may also be influenced by other confounding variables that need to be investigated.

While the newer focus on survivorship issues has led to a more systematic study of chemotherapy-related cognitive impairments (CRCIs), few studies have examined these breast cancer (BC) patients prior to chemotherapy. Older studies were mostly cross-sectional with the whole population being months to years removed from chemotherapy-

treatment [4-7]. Longitudinal studies following patients from diagnosis to treatment and beyond have only begun [8], yet these papers do not carefully examine suspected variables that may be affecting BC patients even before they begin treatment. First, a new diagnosis of a disease and all its associated obstacles are stressful events which could elicit both acute and chronic stress responses via hypothalamic-pituitary-adrenal (HPA) activations. Such increased HPA activity could be subjecting the patients to the negative effects of cortisol on the body and the brain. Also, uncontrollability of one's life is a significant factor in eliciting a stress response [9], and a cancer diagnosis often leads to this feeling. Second, lingering side-effects of surgery and anesthesia administration [2] could be manifesting in patients before chemotherapy, and could additionally cause patients to be more susceptible to chemotherapy agents. Third, previous research has demonstrated that increased cytokine activity found in cancer patients can affect cognitive abilities [3]. Finally, there are also factors such as depression, anxiety and estrogen levels/menopause status that should be considered to produce a complete picture of the BC patient before treatment begins, as well as the role these factors play during and after chemotherapy.

Overall, a pre-treatment patient profile is required in order to gain a clearer baseline status. This would highlight if a BC patient was cognitively impacted by the situation and thus require additional support for decision making. Such study would also limit potential carry-over effects of confound variables that may be mistakenly attributed to side effects of chemotherapy. This would also indicate potential variables that are already manifesting at baseline and whose effects are exacerbated by chemotherapy action. Exposing potential baseline differences is also important since these patients may be vulnerable even prior to chemotherapy, in a time when many decisions must be made. If processes related to executive functioning are affected pre-chemotherapy, the decision process itself is somewhat compromised, which adds to the already demanding pre-chemotherapy situation.

Thesis Breakdown:

The current thesis begins with a comprehensive review paper on CRCIs and the neuroimaging literature. Studies are grouped according to imaging modality (anatomical vs. functional data) as well as subdivided into specific imaging tools and techniques (voxel based morphometry, diffusion tensor imaging, EEG, PET, fMRI). This is followed by a detailed section listing and justifying concern for possible confounding variables that warrant attention in future investigations and could already be manifesting before treatment begins. Finally, this review presents suggestions concerning the choice of control groups and imaging tasks, as well as the challenges of translating current experimental data to clinical practice.

The first original paper investigated neuroanatomical differences between BC patients and sex-, education- and age-matched healthy controls before chemotherapy began. Anatomical assessments were completed using MRI voxel-based morphometry (VBM) tools. Many of the confound variables presented in the above review paper were applied as covariates in the analyses. Neurostructural MRI studies in cancer populations are most prominent and have the most current potential for experimental to clinical applications. Yet, little examination of brain structure in cancer patients (except for brain tumours) before chemotherapy has taken place. This anatomical study applied both whole brain and region of interest (ROI; based on regions reported in post-treatment studies) group comparisons of grey matter and white matter. Major findings focus on group differences in white matter between BC patients and controls.

The second original paper investigated working memory capacities; a common cognitive sequelae reported by post-chemotherapy patients and has been most widely studied in the current literature [5, 10, 11]. Again, few pre-treatment investigations concerning memory abilities in BC patients have been completed. In particular, this study examined neurofunctional differences related to working memory between BC patients and controls, prior to chemotherapy. fMRI data collected during completion of a Visuospatial N-back task were analyzed by whole brain and ROI group comparisons. Major findings revealed differences between BC patients and controls concerning the

neural activation patterns related to working memory. As well, it highlights the need for diverse analysis types such as flexible factorial designs taking into account rest conditions as well as within-group regressions along factors such as errors of commission.

The third and final original paper investigated response inhibition deficits in BC patients compared with controls. Response inhibition is a component of executive functioning which is highly related to successful working memory. While decreased response inhibition capacities have been suggested in the post-chemotherapy population [7, 12], this has not been systematically studied using an fMRI paradigm, either pre- or post- chemotherapy. The present study examined neurofunctional differences related to response inhibition between BC patients and controls, prior to chemotherapy. fMRI data collected during completion of a Go Nogo task were analyzed by whole brain and ROI group comparisons. Major findings revealed functional differences related to response inhibition between BC patients and matched controls. Application of diverse analyses lead to a better understanding of group differences: confounding variables were added as covariates in group comparisons, the effects of the rest blocks compared to the active tasks were considered and within-group variability along confounding variables were investigated through regression analyses.

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Chapter 2

Title:
Opening up the window into "Chemobrain"- A neuroimaging review.

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Abstract:

Modern medical cancer interventions are allowing for greater survival rates. A chemotherapy side-effect, where patients report difficulty during performance of familiar cognitive tasks, is named *chemo fog* or *chemobrain*. Traditional neuropsychological assessments may not be sensitive enough to detect these subtle chemotherapy-related cognitive impairments (or CRCI). The advent of MRI and fMRI tools, as well as other imaging techniques, has helped uncover a neural basis for these reported cognitive impairments. This paper offers a review of neuroimaging investigations in chemotherapy-treated human cancer patients, with a particular focus on breast cancer patients. Additionally, despite the assumption given by the *chemo fog* or *chemobrain* label, chemotherapy is not the only causative agent of these reported deficits, and other factors will be considered. Throughout the paper, suggestions are made on how to more systematically and comprehensively study cognitive changes in chemotherapy-treated cancer patients. Together these suggestions may yield better long-term quality of life outcomes as they could lend support to patients' self-reports, as well as reveal target brain regions being affected by chemotherapy.

Keywords:
Chemotherapy; cognitive impairment; magnetic resonance imaging (MRI); Electroencephalography (EEG); Positron emission tomography (PET).

Highlights

- Neuroimaging has revealed differences between chemotherapy patients and controls.
- Systemic study of the effects of pre-chemotherapy confounding factors is necessary.
- Consistent control group and imaging task choices will help validate prior results.
- Test-retest will aid in translation from research to clinical practice.
- Multi-factorial and -modal studies will clarify the nature of these impairments.

1. Introduction

Recent advances in the diagnosis and treatment of cancers are allowing more patients to achieve complete physical recovery. With this positive trend has come growing concern about the long term side-effects of treatment. Many patients report experiencing cognitive changes while undergoing chemotherapy. In some patients, these changes persist for years post-treatment, and can seriously affect their quality of life as well as that of family and friends. Patients have coined terms like *chemo fog* and *chemo brain* to refer to these changes, indicating their assumption that chemotherapy is the causative agent.

However, results of objective neuropsychological assessments do not always corroborate the deficits reported by patients, and thus such cognitive deficits have historically been dismissed as a consequence of stress alone. This can lead to further patient frustration as they do not feel justified in their complaints and continue to suffer without confirmation of their impairment. One must keep in mind that even subtle changes may have significant functional implications for persons confronting high cognitive demands. Over the last decade, several studies have been conducted in cancer patients to investigate the effects of chemotherapy on cognition, most finding that chemotherapy-treated patients perform more poorly on neurocognitive tests than non-exposed controls (Ahles et al., 1996; Ahles et al., 2002; Bender et al., 2006; Berglund et al., 1991; Brezden et al., 2000; Castellon et al., 2004; Gottschalk et al., 2003; Hurria et al., 2006; Jenkins et al., 2006; Mar Fan et al., 2005; Schagen et al., 1999; Scherwath et al., 2006; Servaes et al., 2002; Shilling et al., 2005; Stewart et al., 2007; Tchen et al., 2003; van Dam et al., 1998; Wagner et al., 2006; Wefel et al., 2004; Wieneke and Dienst,

1995). Even prospective studies (Bender et al., 2006; Collins et al., 2009; Jansen et al., 2008; Jenkins et al.., 2006; Quesnel et al., 2009; Shilling et al., 2005; Stewart et al., 2007; Wefel et al., 2004; Wefel et al., 2010), which additionally include pre-treatment baseline testing and closely matched controls, reveal subtle cognitive declines after chemotherapy exposure. (Of note, two studies reported no increase in the frequency of cognitive impairment in chemotherapy-treated BC patients compared to healthy individuals (Donovan et al., 2005; Scherwath et al., 2006).)

The estimated prevalence of cognitive deficits in chemotherapy treated populations is highly variable with a range from 17% to 75% reported across studies (Correa and Ahles, 2007). Such variability makes it difficult to convince those outside the patient population of the reality of cognitive impairments found in cancer patients undergoing chemotherapy. The considerable variability in results from one study to the next are due to differences in key study design factors including: 1. sample size (many studies use only a small number of patients), 2. differences in the nature of the neuropsychological battery used (e.g. targeted tests or complete battery) resulting in differential sensitivity to subtle cognitive changes, 3. increased sensitivity of computerized testing in conjunction with pencil and paper assessments, 4. nature of the control group (example: healthy controls versus non-chemotherapy patient group), 5. definition of cognitive impairment adopted, 6. effects of anesthesia on cognition for patients who also underwent surgery (Monk et al., 2008), 7. stress of cancer diagnosis and treatment, 8. existence of pre-treatment differences in cognition between BC patients and controls (Cimprich et al., 2010), 9. possible negative effects of endocrine treatment on cognition (Quesnel et al., 2009), and 10. data analytic methods used, in particular,

whether impairment is defined at the group or individual level and, in the case of longitudinal studies, whether or not the analyses control for practice effects associated with repeated testing. These factors must be systematically controlled in future studies if progress is to be made in understanding the effects of cancer treatments on cognition. There are many reviews on neuropsychological assessments in cancer and chemotherapy-treated patients. For further and more detailed readings on the neuropsychological findings in chemotherapy-treated patients, please refer to the following most recent reviews: (Vardy and Tannock, 2007; Correa and Ahles, 2008; Wefel et al., 2008).

The following review article will review the limited imaging research on chemotherapy-related cognitive impairments (or CRCI) in adult samples only. There will be a particular focus on women with breast cancer since most CRCI imaging investigations are conducted in this population. Both structural and functional imaging studies will be described and synthesized in separate summary tables, possible confounding variables to be considered in future studies will be discussed, as well as the need for better control groups and the challenge of translating current data to clinical practice.

2. Findings from imaging studies

Even with use of increasingly sophisticated performance-based assessments, there is still the concern that subtle chemotherapy-induced deficits are not being recognized or acknowledged. Additionally, the neural structures and/or circuits that are being affected by chemotherapy treatment are still relatively unknown. In an attempt to provide empirical evidence for chemotherapy-related cognitive impairments (or CRCI), neuro-imaging tools are increasingly being used to examine the effects of chemotherapy on the

brain and cognition (0; Ferreira et al., 2009; Raffa, 2010). Application of such tools could help uncover a neural basis for the subtle cognitive deficits in affected patients. However, there are only a handful of imaging studies that have examined the CRCI phenomenon and thus further brain imaging research is required.

2.1. Structural neural changes

2.1.1. Advantages

There are many techniques that can help uncover structural changes in the brain. First, magnetic resonance imaging (MRI) can produce quality two- or three- dimensional images of brain structure without the use of ionizing radiation or radioactive tracers. The detection mechanisms of this technique are so precise that changes in neural structures can be detected over time (Filler, 2009). This technique can also create images of both surface and subsurface structures, which can then be viewed as slices in any direction (top to bottom, side to side, front to back). The two main techniques applied to uncover tissue density (ex: grey matter) or volume differences are either manual segmentation protocols or voxel-based morphometry (VBM) tools. Manual segmentation protocols are highly specific and there are validated sets of instructions concerning individual brain regions (eg. Hippocampus, amygdala) which require extensive training as well as inter-researcher reliability. Meanwhile, voxel-based morphometry (or VBM) techniques are a widely used automated technique that divides the brain into grey, white and cerebrospinal fluid (CSF) and can provide tissue density and/or volumes for whole brain or region of interest investigations.

Second, Diffusion MRI techniques allow for another structural assessment of the brain, producing in vivo images of biological tissues weighted with the local micro-

structural characteristics of water diffusion. Within this, there are two distinct classes of application. Diffusion weighted MRI, or DWI, provides information about damage to parts of the CNS. This technique measures the rate of water diffusion at a specific location and is best applied in a tissue where the diffusion rate typically appears to be the same when measured along any axis (e.g.: grey matter). The second diffusion technique is called diffusion tensor MRI, or DTI, which provides information about connections among brain regions. This technique capitalizes on the magnitude and direction of diffusion through a particular internal structure. Water will diffuse more in the direction aligned with the fibers and less when perpendicular to the preferred direction. This technique is therefore best when examining tissues with an internal fibrous structure such as white matter tracts. An extended application to this last technique is the ability to derive neural tract direction information, with the assumption that the diffusion within each voxel is homogeneous and linear. A particular measure called fractional anisotropy (FA) assesses whether water movement in a certain voxel is unrestricted (water movement can occur along all axes) or restricted (water movement only occurs along 1 axis). A FA score closer to 1 means greater integrity of the tissue in that particular voxel (or restricted water movement) while a score closer to 0 means less integrity (or unrestricted water movement). Additionally, DTI techniques can be used to infer the white matter connectivity of the brain, otherwise known as tractography, which reveals brain region connections. Overall, both diffusion techniques can help uncover structural neural changes over time and possible differences between groups concerning neural tissue integrity. These are a measure of the efficiency of neural communication. (For a review on Diffusion MRI techniques, please refer to Johansen-Berg and Behrens (2009).)

2.1.2. Review of anatomical findings

Structural brain changes have been associated with chemotherapy in cancer patients (Brown, 1998). Cerebral white matter is vulnerable to neurotoxins, such as chemotherapy agents, and use of MRI techniques has increased recognition of white matter changes related to drug intake (ex: leukoencephalopathy in leukemia patients after taking immunosuppressive drugs). Furthermore, these white matter changes are strongly correlated with grey matter volume changes. Overall, it is thought that both white matter and grey matter changes can be reversible. The regions reported in the structural studies outlined below are also reported in Tables 1.1 and 1.2[a].

2.1.2.1. Anatomical MRI

One of the first studies by Brown et al. (1998) prospectively assessed white matter volume changes induced by high-dose chemotherapy using anatomical MRI techniques. Eight patients with advanced breast cancer were scanned prior to beginning chemotherapy, with six patients receiving induction chemotherapy. All patients completed the chemotherapy regimen, but only four patients were available for the subsequent 1, 3, 6, 9 and 12 months post-chemotherapy follow-ups. Results indicated no structural abnormalities at baseline. However, three of the four patients scanned at the 3 month post-chemotherapy time point showed a progressive increase in abnormal white matter volume, with maximal volume changes ranging from 73-166 cm^3. These volume changes subsequently stabilized from 6 to 12 months after chemotherapy completion. These investigators also conducted brain MR spectroscopy (MRS), a technique which is used to measure metabolite levels in different body tissues (in other words, it provides the opportunity to collect biochemical information non-invasively). Results from the neural

MRS showed little to no change in metabolic ratios from baseline to the post-chemotherapy follow-ups. A small transient decrease in *N*-acetylaspartate compared to creatine, which occurred post-treatment was suggested (decreases in *N*-acetylaspartate are commonly reported in MRS studies as a reduction in neuronal integrity). Overall, the authors conclude that there are treatment-related white matter changes occurring with high-dose chemotherapy regimens, which progressively accumulate until around 6 months but are not accompanied by persistent neurologic symptoms. Minimal disturbance of the biological marker *N*-acetylaspartate, as indicated by the MRS, may partly explain this good neurological outcome. This study was the first to explore chemotherapy-related structural changes in the brain using a prospective design. Although the small patient sample resulted in limited power to test assumptions, this study was important because it led the way for subsequent anatomical studies of chemotherapy-related brain changes.

Saykin et al. (2003) recruited 12 chemotherapy-treated survivors of breast cancer or lymphoma within 5 years of completing treatment as well as 12 healthy matched controls. These patients completed high-resolution anatomical MRI, and voxel-based morphometry (VBM) techniques were applied to quantify both white and grey matter volumes of various brain regions. The chemotherapy patients showed local bilateral volume reduction of neocortical grey matter as well as diffuse cortical and subcortical white matter volume reductions compared to controls.

Another VBM assessment of breast cancer patients was completed by Inagaki and colleagues (2007). Instead of assessing volume differences between controls and patients at only one time point, they assessed brain volume changes at 2 different time points.

Additionally, they controlled for the effects of having cancer itself. At year 1 (range 3-15 months post-chemotherapy), the scanned population included 51 adjuvant chemotherapy-treated breast cancer survivors as well as 55 breast cancer survivors who received surgical treatment only without chemotherapy. At year 3 (range of 27-39 months post-chemotherapy), they scanned 73 breast cancer survivors who had received adjuvant chemotherapy (some participants had also done scan 1, others were new study recruits), and 59 survivors who had received local therapy only. These VBM-processed brain images were referenced to images of healthy controls (year 1, n=55; year 3, n=37). At the one-year time point, the chemotherapy patients were found to have smaller volumes in key areas involved in cognitive processing, including grey matter losses in the right prefrontal cortex and para-hippocampal gyrus and white matter decreases in the bilateral middle frontal gyri, left para-hippocampal gyrus, left precuneus and right cingulate gyrus. Additionally, there was a strong positive correlation between volume loss and performance on tests of attention and memory (Wechsler Memory Scale Revised). Interestingly, these results were no longer evident at the 3-year follow-up scan. When pooling the cancer groups and comparing to healthy controls, there were no regional differences at both the 1-year and 3-year scans. A serious weakness of this study was the failure to consider the effect of adjuvant endocrine therapy, given that anti-estrogen therapy has also been implicated in cognitive dysfunction in breast cancer patients (Castellon et al., 2004). Another limitation, shared by the Saykin et al. (2003) study presented above, is the lack of a pre-chemotherapy baseline, which makes it impossible to assess whether the observed abnormalities were present prior to treatment. Despite

these limitations, the Inagaki study is important since it was the first to suggest, using an adequate sample, a transient effect of chemotherapy on brain structure.

A more recent study (McDonald et al., 2010) applied the first prospective design to studying CRCI using structural imaging. The researchers recruited 17 breast cancer patients who were receiving chemotherapy treatment, 12 breast cancer patients who were not treated by chemotherapy, and 18 matched healthy controls. These patients completed high-resolution anatomical MRI at baseline (after surgery but before beginning treatment), 1 month after chemotherapy completion and 1 year later. VBM techniques were applied to quantify whole brain grey matter volume. Results indicated that there were no group differences at baseline. Both cancer groups showed grey matter decline at the 1 month mark compared to controls. Chemotherapy-treated patients revealed grey matter reductions in bilateral middle frontal gyri and left cerebellum, while non-chemotherapy patients had decreased volumes in the right cerebellum. At 1 month after chemotherapy, the chemo-treated group showed decreased grey matter in bilateral frontal, temporal and cerebellar regions as well as the right thalamus compared to baseline. In this same group, recovery of grey matter volume is suggested in the bilateral superior frontal, left middle frontal, right superior temporal and cerebellar regions at year 1 compared to at 1 month. Meanwhile, in this same condition, decreased grey matter volumes did not recover in the right thalamus, right medial temporal lobe, left middle frontal gyrus, right precentral gyrus, right medial frontal and right superior frontal gyri as well as bilaterally in other regions of the cerebellum. These authors even attempted to control for any effect of surgery by using the “days between surgery date and baseline MRI scan” as a covariate in the group comparisons. Adding a covariate attenuated the

statistical model, but the results remained unchanged. The authors additionally applied other factors as covariates to reveal possible confounding effects and none were significant. This indicates that some speculated confounding factors do not influence grey matter volume. However, much larger groups are essential to tease-out the effects of any "contributing" factors being applied as covariates. Overall, this study is strong since it adopts the first prospective design of a structural assessment of the chemotherapy brain. Additionally, the use of 3 groups: (BC patients with chemotherapy, BC patients without chemotherapy and healthy matched controls) is an asset. This grants simultaneous comparisons of chemotherapy-treated patients to both "healthy" controls as well as BC patients without chemotherapy, the latter controlling for factors such as the presence of cancer, or stress of a cancer diagnosis. However, one must take into account that BC patients who did not receive chemotherapy underwent a different treatment which could present a confound itself, highlighting the importance of having a healthy control group in addition to cancer groups in such studies. Finally, the decreased grey matter volumes reported in this study overlap with those reported in previous structural studies, notably in the frontal and temporal regions (Inagaki et al., 2007). Interestingly, this was the first study to report anatomical changes in cerebellar volumes, corroborating with studies revealing functional changes in this region (Silverman et al., 2007).

2.1.2.2. Diffusion MRI

Two studies have investigated cognitive impairment and brain connectivity in a chemotherapy-treated population using Diffusion Tensor Imaging (DTI) tools. Abraham et al. (Abraham et al., 2008) applied a fractional anisotropy (FA) technique to measure white matter integrity in the genu (anterior) and splenium (posterior) parts of the corpus

callosum that connect the cerebral hemispheres. They recruited 10 chemotherapy-treated breast cancer patients who reported cognitive problems, although unfortunately complaints were not formally assessed. Patients were enrolled in the study for an average of 22 months (range: 3-32 months) after therapy completion. They also scanned 9 healthy, age- and education-matched women as controls. Participants completed a digit symbol test which assessed processing speed. Results showed slower processing speed in chemotherapy-treated participants. Chemotherapy treatment was also correlated with lower FA scores in the genu of the corpus callosum, indicating that adjuvant chemotherapy affects normal-appearing white matter integrity in this area. Moreover, this decreased white matter integrity was significantly correlated to cognitive deficits reported by chemotherapy-treated breast cancer patients. The breast cancer patients in this study were all on anti-estrogen agents at the time of scanning but, like the anatomical studies above, the effects of endocrine treatment were not considered in the analysis and there was no pre-chemotherapy baseline. However, this study was significant in that it was the first to indicate changes in white matter integrity following chemotherapy, as well as a correlation between decreased performance and this white matter change.

A more recent study by Deprez et al.(2010) examined cerebral white matter integrity in 17 BC patients post-chemotherapy compared to 10 non-chemotherapy treated BC patients and 18 healthy matched controls. These researchers used DTI in combination with comprehensive neuropsychological testing and a self-report questionnaire concerning cognitive failure. The authors applied FA techniques but additionally measured mean diffusivity (MD), which quantifies water diffusion within a tissue and is affected by the cellular size, shape and integrity of that tissue (Pierpaoli et al., 2006). MD

values decline with increasing tissue barriers, such as cell membranes and myelin sheaths (Basser and Pierpaoli 1996). Water runs into less obstruction with edema, demyelination and axonal loss (Iannucci et al., 2001; Stevenson et al., 2000) and can travel further, thus a larger value is indicative of more free water and decreased tissue integrity. Deprez et al. (2010) reported that, compared to non-chemotherapy patients and healthy controls, chemotherapy-treated BC patients showed decreased FA in frontal and temporal white matter tracts, as well as increased MD in frontal white matter. A voxel-based correlation analysis between FA scores and individual neuropsychological test scores revealed significant correlations between neuropsychological tests (attention and processing speed) and FA scores in the temporal and parietal white matter tracts. Furthermore, self-report cognitive failure questionnaire (CFQ) scores were also negatively correlated with frontal and parietal white matter. Overall, chemotherapy-treated BC patients performed worse on the neuropsychological tests than the other two groups, with 7 of these patients classified as clinically impaired on follow-up analysis. Compared to controls, these "impaired" chemotherapy patients had lower FA values than their "unimpaired" chemotherapy-treated counterparts. Like Abraham et al. (Abraham et al., 2008), these authors suggest that micro-structural white matter changes or abnormalities may underlie reported cognitive dysfunctions found in chemotherapy-treated cancer patients. However, again, such conclusions are incomplete as the effects of endocrine treatment were not considered and there was no pre-chemotherapy baseline data.

2.1.3. Limitations of anatomical assessments

Anatomical assessments of the brain using MRI can indicate anatomical neural changes over time, as well as indicate differences between groups. However while such a tool can uncover structural differences, it fails to provide information about how the brain is actively functioning at the time of imaging. This is particularly important since one can suspect that with anatomical changes, there will be related functional changes.

2.2. Functional neural changes

2.2.1. Advantages

Functional neuroimaging gives insight into the working brain through a variety of mediums. For example, it can assess the brain's electrical activity, or measure localized changes in cerebral blood flow related to neural activity (referred to as activations).

First, an electroencephalography (or EEG) is an imaging technique that is used to measure electric fields in the brain via use of electrodes carefully placed on the scalp. Whenever there is electrical activity in brain regions near the surface, this can be recorded by the electrodes. The P300 is one of many different waves that can be measured as an event-related potential (ERP), being elicited by infrequent task-relevant stimuli and dependant on the participant's reaction to the stimuli. The P300 wave is typically measured most strongly by electrodes at the parietal lobe (or Cz scalp location) (Donchin and Coles, 1988; Kok, 2001). The magnitude, timing and topography of an ERP are metrics of cognitive function in cognitive tasks. EEG offers high temporal resolution (on the order of milliseconds) despite poor spatial resolution.

Second, Positron Emission Tomography (or PET) uses radioactively-labeled and metabolically-active chemicals (injected into the bloodstream) to view metabolically

active brain regions during a cognitively engaging task. The emissions from these radioactive chemicals are detected by the sensors in the scanner and then this data is computer processed to reveal their distribution throughout the brain. The most commonly used ligand to examine neurotransmitter activity is a form a glucose, essentially the "body's fuel", called Fludeoxyglucose (or FDG). The greatest benefit of using PET is the ability to show both blood flow changes as well as brain tissue metabolism (in particular both oxygen and glucose) in a "working brain". As well, it is particularly useful in cases where damage is diffuse (example: early Alzheimer's disease), with few apparent changes to gross brain volume and structure.

Third, Functional MRI (fMRI) techniques also provide a window into the working brain as it is a non-invasive neuroimaging technique that localizes brain function in response to motor, sensory or cognitive tasks. The basis for fMRI is that increased neural activity in a region is accompanied by a substantial increase in local blood flow rich in oxygen (leading to a reduction of deoxyhemoglobin), which results in an increase of magnetic resonance (MR) signal intensity. This is called the blood oxygen level dependent (BOLD) effect (Arthurs and Boniface, 2002; Logothetis and Wandell, 2004) allowing for safe brain imaging with no injection of a contrast material and no exposure to ionizing radiation (as in the case in PET). This technique offers excellent spatial resolution (2-3 millimeters) and moderately good temporal resolution (slower than EEG but faster than PET). Most importantly, its reliability has been well-established (Kwong et al., 1992; McAllister et al., 2001; Smith et al., 2004a; Smith et al., 2004b; Smith et al., 2006; Wishart et al., 2004). This tool has already successfully been applied to clinical

questions of mild cognitive impairment in other disorders (such as MS and mild brain injury) (McAllister et al., 2001; Wishart et al., 2004).

2.2.2. Review of functional findings

In addition to the small number of structural imaging studies, a limited number of functional imaging studies have assessed neural changes in chemotherapy-treated cancer patients. The regions reported in both PET and fMRI studies outlined below are also reported in Table 2[b].

2.2.2.1. Electroencephalography (EEG)

There are EEG investigations of the chemotherapy-treated brain. Due to the high temporal resolution of EEG, this is a useful imaging technique for quantifying subtle differences in timing of neuronal firing. This electrical activity can be extracted and can partially explain subtle cognitive differences precipitated by chemotherapy treatment.

The first EEG study (Kreukels et al., 2005) examined 26 BC patients with adjuvant CMF chemotherapy (CMF regimen: Methotrexate and 5-Fluorouracil) as well as 23 stage 1 BC patients who were not chemotherapy-treated (they received radiation treatment) during an information processing task. This task had 3 conditions: 1. determine the direction of the double arrowhead (easy discrimination task), 2. press the button with the hand on the same side as the stimulus presented on the screen (easy) or the opposite side (difficult) to examine spatial compatibility and 3. use only the index finger to respond (easy) or respond with a sequence of button presses using more than one finger (difficult). The authors examined event-related potentials (ERP) at Cz, specifically the P300 peak (both amplitude and latency) as well as task reaction time.

Participants were tested at 5.1 years post-chemotherapy and 3.6 years post-radiation, respectively. Results indicated that there were no performance differences between groups for errors or reaction times. Chemotherapy-treated BC patients revealed shorter P300 latency as well as decreased amplitude of the P300 compared to non-chemotherapy BC patients. Interestingly, chemotherapy patients reported more daily cognitive deficits compared to non-chemotherapy patients, yet this effect was not significant between the groups. The authors suggest that the decreased P300 amplitude in chemotherapy patients indicates a decrease in the timing of mental processes, as smaller P300 amplitude is associated with more difficulty performing a task. This study was the first to explore the chemotherapy-treated breast cancer patient using EEG technology while participants completed a cognitive task. Unfortunately this was a cross-sectional study design and thus pre-treatment assessments of BC patients were not performed. Additionally, the authors reported difficulty with the complexity of the task for participants in both groups as the successful completion or mastering of the complex response pattern was very difficult. If participants felt unable to complete a task condition, this may lead to increased stress or error rumination and could be considered a confounding factor in interpreting the results of the study. It is then possible that the decreased P300 observed in the chemotherapy-treated patients when compared to radiation-treated patients could be due to changes, such as adopting a negative attentional bias or increased rumination behaviour that could be present post-treatment. Additionally, one must also consider that perhaps the radiation-treated patients benefited more from the extensive training that took place before the ERP measurements, which translated into this group not requiring as large an energy expenditure related to information processing during the measured task

compared to the chemotherapy-treated group. These statements need to be considered for the numerous other functional studies in this population that are explained below (EEG, PET, fMRI).

A second EEG study (Kreukels et al., 2006) measured 29 adjuvant BC patients treated with chemotherapy (17 high dose and 12 standard dose) as well as 23 early-stage BC patients without chemotherapy-treatment, at 4 years post-treatment completion. The authors applied the same information processing task discussed above and examined task reaction times and ERPs. Results indicated that performance accuracy did not differentiate between groups, however when applying age as a covariate, the chemotherapy-treated patients (combined high and standard doses) showed slower reaction times compared to non-chemotherapy controls. Additionally, high dose chemotherapy-treated patients revealed decreased P300 amplitude compared to controls, while there were no significant differences between patients who received standard chemotherapy doses compared to non-chemotherapy controls. Interestingly, self-reported cognitive complaints did not correlate significantly with any of the behavioural or neuropsychological measures. Overall, this study suggests that the reduced P300 amplitude seen in the high dose chemotherapy group indicates a problem with allocation of processing resources. This study is particularly important since it noted that only the high dose population revealed a decreased P300 amplitude. This is the first study to really indicate that different chemotherapy regimens may have differential effects on the P300 component, and therefore on cognitive functioning. However, these results suffer from a lack of power in the chemotherapy sub-divisions and a larger study is required to significantly reproduce these results. As with the above study (Kreukels et al., 2005), the

failure of a task condition to be completed or mastered by many patients, regardless of treatment, must be considered as a possible confound. This must be noted, even if this task condition was removed from the analysis since there could be a carry-over effect. As well, since there were no healthy matched controls, one cannot speculate on the reason behind the increased level of failure in this complex task. Obtaining P300 data from healthy controls would have also strengthened this study, as would a prospective study design.

The most recent EEG paper from the same laboratory (Kreukels et al., 2008) further teased out the differential effects of different chemotherapy regimens on possible cognitive impairments and P300 amplitude and latency changes. The authors applied an auditory oddball task at 3-6 years post-treatment in 4 groups: 53 chemotherapy-treated BC patients (12 high dose, 17 standard dose and 24 CMF treatment, the later comprised of a combination of cyclophosphamide, methotrexate and 5-fluorouracil) as well as 23 early stage BC patients who did not receive chemotherapy treatment as the control group. Participants were instructed to listen to 2 different tones: 1000Hz occurring 80% of the time and 2000Hz occurring the other 20% and asked to count the number of times the 2000Hz tone is heard. EEG measurement focused on the N100 and P300 components (amplitude, latency). Results revealed no group performance differences in the number of tones counted, as well there were no significant differences in N100 latency and amplitude. Meanwhile, the CMF group revealed shorter P300 latency compared to high-dose chemotherapy patients. Chemotherapy-treated patients combined revealed a lower P300 amplitude compared to non-chemotherapy treated patients. These results lead the authors to suggest that early perceptual processes (N100) are intact in the chemotherapy-

treated groups. Meanwhile P300 amplitude and latency were revealed as being different between groups in the oddball task. This supports the notion that chemotherapy treatment can have effects on brain functioning and that different regimens can cause differential effects. The authors also addressed the fact that shorter P300 latency seen in the CMF group is surprising since most cognitive disorders show a lengthening effect. Similarly to conditions such as ADHD and OCD who reveal shorter P300 latency, this could be the result of cognitive speeding due to decreased response inhibition capacities. The most important contribution from this paper however is the revelation that heterogeneous treatment groups, as seen often in chemotherapy and cognition studies, do not extract intricate differences that varying regimens provide. For example, in a heterogeneous group, 2 subgroups could experience opposite effects in the brain (ex: lengthening and shortening of the P300 latency) which would erroneously lead to the conclusion that there is no effect. This further division of the chemotherapy-treated population is essential, but creates challenges in an already challenging recruitment process as much larger sub-groups would be a necessity and there would be even more issues with control matching. While this cross-sectional study is a first and very important step in further investigation of the differential effects of regimens, a prospective study design would make it much stronger and investigate possible baseline differences between groups even before treatment.

Finally, one prospective study using EEG tools is currently underway under the supervision of Dr. Halle Moore (Cleveland Clinic's solid tumor oncology unit). This study is comparing eight early-stage breast cancer patients with matched controls using EEG tools and cognitive tests at 3 time points (before, during and after chemotherapy).

While this participant number is very small, it will be the first prospective study using EEG tools investigating the chemotherapy-treated BC patient's brain.

2.2.2.2. Positron Emission Tomography (PET)

One PET study of CRCI has been published to date. Silverman et al. (2007) recruited 16 long-term chemotherapy treated breast cancer survivors with memory problems (minimum 5 years post-chemotherapy; 11 patients also had tamoxifen) and 8 non-chemotherapy-treated controls (5 were BC survivors and 3 never had BC). A previously acquired standard reference group of 10 healthy controls who underwent a [F-18] fluorodeoxyglucose-PET study were also used. Investigators used [O-15] PET to study blood flow during a delayed-recall word memory task. Delayed recall questions activated a larger portion of the inferior frontal cortex in chemotherapy patients compared to untreated patients. Additionally, [F-18] fluorodeoxyglucose-PET was used to assess resting metabolism between chemotherapy treated patients and controls. Results demonstrated that chemotherapy patients showed lower resting brain metabolism, particularly in the left inferior frontal gyrus and in the contralateral cerebellum. Furthermore, the more impaired the performance on the ROCF delayed recall task, the lower the resting metabolism in frontal cortex. (NB: The inferior frontal cortex is already less metabolically active under healthy circumstances.) Also, basal ganglia metabolism was decreased in patients treated with both chemotherapy and tamoxifen. Overall, study results suggest that the frontal cortex has to work much harder to successfully perform the task in the chemotherapy-treated subjects. While, this study does not have a pre-chemotherapy baseline assessment, it does consider the effects of combination treatment in the analysis (chemotherapy + tamoxifen). This study (Silverman et al., 2007) is one of

the first to indicate potential functional neural changes in chemotherapy treated patients. These alterations in brain activity are an indication that the brain is working differently, either through new neural recruitment or compensation, to accomplish the same performance (Kwong et al., 1992; McAllister et al., 2001; Smith et al., 2004a; Smith et al., 2004b). These neural changes may alter subjective experience of cognitive efficiency, such that cognitive activity seems to take more effort and result in greater fatigue, and thus may underlie patients' cognitive complaints. This might explain why many chemotherapy-treated breast cancer (and other cancer) patients fail to return to premorbid levels of social/occupational functioning despite excellent physical recovery. This would indeed affect quality of life.

2.2.2.3. Functional Magnetic Resonance Imaging (fMRI)

An interesting case study by Ferguson et al. (2007) involved a pair of monozygotic twins, only one of whom had received chemotherapy treatment for breast cancer. While no significant performance differences were revealed on memory and executive functioning assessments, the chemotherapy-treated twin reported subjective cognitive complaints. Additionally, structural brain differences between the twins were assessed (anatomical MRI) and revealed that the chemotherapy treated twin had greater white matter lesion volume than the untreated sibling. Finally, fMRI during a working memory task revealed larger and more diffuse areas of activation in bilateral frontal and parietal regions in the chemotherapy treated twin. While the small population size is an obvious drawback, the differences between these two genetically identical individuals may reflect the effects of chemotherapy on one member of the pair. It also suggests that a larger scale twin study could reveal much about the neural underpinnings of CRCI,

particularly if a pre-chemotherapy baseline assessment were included in the study design. However, recruiting such a population would be ambitious and would most likely take years.

Using fMRI during a working memory task, Saykin et al. (2006) imaged fifteen chemotherapy-treated breast cancer patients, seven local therapy only breast cancer patients and seven healthy controls. Groups were matched for age, gender (all women), education, and estimated baseline intellectual ability (according to education and Barona full scale IQ). Working memory was assessed using an auditory N-back task with variable processing load requirements (0, 1, 2, and 3-back conditions). This was the first prospective functional neuroimaging study to establish an appropriate baseline by scanning participants prior to beginning chemotherapy or radiation treatments. Repeat scanning (time 2 scan) was conducted within one month of the completion of the chemotherapy treatment and at the same time point for non-chemotherapy exposed participants. The authors reported no group differences in task performance; they observed the expected main effect of working memory load on performance across groups and time. All groups showed expected activation patterns at the most challenging load (3-back), with bilateral activations of frontal, parietal, and cerebellar regions (baseline and time 2 scan). However, fMRI results from the time 2 scan revealed that chemotherapy-treated patients showed increased activation in posterior frontal and parietal regions compared to controls and local therapy patients and less bilateral activity in more anterior frontal regions. Therefore, as seen in fMRI studies of other disorders associated with mild cognitive impairment, compensation may allow performance levels to be functionally maintained in spite of changes in brain activation. These findings

illustrate that the cognitive changes associated with chemotherapy may be too subtle to reliably detect with standard neuropsychological assessments and underscore the importance of and potential for using fMRI to elucidate underlying neurobiological changes. In addition to highlighting the benefits of fMRI, Saykin et al. (2006) also demonstrated a practice effect whereby all groups showed improved 3-back task performance at the time 2 scan. Interestingly, this improvement over time correlated with increased bilateral prefrontal activations in all groups. Without a control group at both time points, this change in activation may have been wrongly attributed to the effects of chemotherapy. This emphasizes the importance of having a matched control group at each testing time point when examining a clinical population.

One study (Kesler et al., 2009) investigated verbal declarative memory, using fMRI, in fourteen BC woman (8 metastatic, 6 locally advanced) who had a history of adjuvant chemotherapy treatment (mean time since last treatment was 3.3 ± 3.3 years, range: 0.5 – 10.3 years). Patients were age and education-matched to healthy female controls. Participants completed salivary cortisol sampling and other questionnaires as distress measures and completed an fMRI adapted verbal declarative memory encoding and recall task while in the scanner. Results indicated that there were no between group differences in any of the distress measures. While both patients and controls revealed similar response accuracy on the encoding component of the task, a trend revealed that patients had faster reactions times. Patients showed less activity in the left superior frontal gyrus extending to the right superior frontal gyrus, bilateral middle frontal gyri and left postcentral gyrus. During the recall component, both groups revealed similar response accuracy yet BC patients had slower reaction times compared to controls.

Patients showed greater activation in right superior temporal gyrus extending to bilateral fusiform, bilateral lingual gyri, left hippocampus, bilateral basal ganglia, right precentral gyrus, right superior and middle frontal gyri, bilateral inferior frontal gyrus, right cingulate gyrus, bilateral insula, bilateral parahippocampal gyrus, bilateral cuneus, bilateral precuneus, bilateral superior parietal lobe and bilateral cerebellum. The authors suggested that quicker reaction times during encoding along with decreased prefrontal activations could be indicative of increased impulsivity in patients compared to controls. Also, while task accuracy is similar for both groups, patients require greater and more wide-spread neural effort than controls when attempting to recall task information- thereby explaining the greater fatigue and frustration reported by post-treatment patients. Chemotherapy regimen type was exposed as contributing to differential patient verbal memory impairments; CMF treated patients showed lower prefrontal cortex activity during encoding compared to ACT treated patients. This study was the first to examine verbal encoding and memory capacities of chemotherapy-treated patients in an fMRI setting, as well as distress variables. Additionally, it demonstrates differential neural activations with varying chemotherapy regimens, which highlights the importance of distinguishing between type of chemotherapy-treatment. Finally, the limitation of a small sample size in this cross sectional design is exacerbated by the rather large span of time since treatment completion, ranging from 6 months to 10.3 years after treatment. While the authors report no relationship between increased time since treatment and improved brain function, one must be careful to collapse short-term and long-term patients into one population, especially when a population is small and subject-driven effects are more probable. As well, many of the BC patients also underwent other cancer-related

treatments such as radiation and tamoxifen administration which are additional confounding variables.

The largest and most recent fMRI study (de Ruiter et al., 2010) examined 19 high dose adjuvant chemotherapy BC survivors (only 16 used in the fMRI analysis) and 15 non-chemotherapy treated BC survivors at 10 years after treatment completion. The authors measured performance on 16 neuropsychological tests as well as BOLD activation and task performance during 2 tasks, applying both age and estimated IQ as covariates. Overall, the chemotherapy group was found to be impaired on significantly more of the 16 neuropsychological tests than the control group. The first fMRI task was an adapted Tower of London task which extracts executive functioning involving planning capacities and the second, a paired-associates task which assesses episodic memory. Results revealed that the chemotherapy group performed significantly worse on only a single neuropsychological test out of 16 and this was the word fluency proficiency test. The fMRI data revealed that the chemotherapy group performed more poorly and quicker on the Tower of London task. Additionally, the chemotherapy group showed hyporesponsiveness of the dorsolateral prefrontal cortex in an ROI analysis, as well as decreased activation in the bilateral posterior parietal cortex in a whole brain analysis. Additionally, this hyporesponsiveness was maintained in the chemotherapy treated group during the paired associates task compared to non-chemotherapy treated survivors in the parahippocampal gyrus in an ROI analysis as well as bilateral lateral posterior parietal cortex, left precuneus, right dorsal striatum, right inferior parietal cortex and left middle temporal gyrus in a whole brain analysis. The authors reported that the chemotherapy-treated group showed borderline significant impairment on recognition memory. Overall,

the authors suggest that high dose adjuvant chemotherapy is associated with long-term cognitive impairments, linking dorsolateral prefrontal cortex hypoactivation with impaired planning behaviour observed in the chemotherapy-treated BC patients. Additionally, the authors indicate that quicker reaction times in patients, along with poorer accuracy could be an indication of increased impulsivity due to impaired attentional abilities. Both tasks, although very different paradigms, revealed parietal hypoactivation, which supports the idea that chemotherapy-treatment induces long-term effects on attentional abilities. This study is the first to examine fMRI data in a patient group 10 years after-treatment, thereby examining long-term negative effects of chemotherapy on cognitive function and linking it with regional brain activity. However, this is a cross-sectional design; a prospective long-term study could have provided more than a snapshot by dynamically showing how the chemotherapy-brain changes from baseline, as well as which neural activity is maintained, recuperated or continuously lost after treatment and over an extended period of time. Additionally, this study could have benefitted from comparisons with a matched healthy control group along with the non-chemotherapy survivor group to control for possible long-term effects related to differential treatments.

2.2.3. Limitations of functional assessments

Although functional neuroimaging provides a view into the working brain, all of these techniques sacrifice either temporal or spatial resolution at the expense of the other. Ideally, a multi-modal imaging study of the CRCI would capitalize on the high sensitivity of both the spatial and temporal resolutions of two different but complimentary tools, for example, a combined EEG/fMRI study. Another important factor to mention concerning

functional imaging studies in this field is the consistent use of small samples. Defining an appropriate sample size is difficult because it is dependent on the scale of the effect being measured- and a prominent issue in this rather new field of study is that exact effects are still unknown. However, for standard neuroimaging studies, one must strive to assess no less than 20 participants (Poldrack, 2009).

3. Multifactorial Approach

3.1. Other factors to consider in the study of CRCI

Research into the biological mechanisms underlying cognitive deficits in cancer patients must address a myriad of potential confounds and thus requires a multivariate, multi-technological approach. One possible factor mediating the impact of cancer and chemotherapy on cognition is stress.

3.1.1. Stress and glucocorticoids

Undoubtedly, a cancer diagnosis gives rise to intense chronic stress for many individuals, especially those who are subjected to prolonged noxious treatments. Stress is routinely measured in CRCI studies through self-report, and this measure correlates highly with complaints of cognitive disturbances (Bender et al., 2006; Castellon et al., 2004; Jenkins et al., 2006; Schagen et al., 1999; Schagen et al., 2002; Shilling et al., 2005; Shilling et al., 2007; van Dam et al., 1998). Stress is associated with increases in glucocorticoids (GCs), such as cortisol due to increased activity of the hypothalamic-pituitary-adrenal (HPA) axis. Increased levels of GCs, which can also result from therapeutic GC administration as part of the immunosuppressive therapy used in cancer treatment, have themselves been shown to affect brain anatomy (Bremner et al., 1999;

McEwen and Magarinos, 1997; Sapolsky, 1985) as well as memory functions such as working memory (Lupien et al., 1999; Taverniers et al., 2010; Oei et al., 2006). It is hypothesized that the hippocampus is particularly vulnerable to glucocorticoid-mediated excitotoxicity due to its high number of glucocorticoid (GC) receptors. In fact, smaller hippocampal volumes are seen in populations with higher stress reactivity profiles suggestive of higher circulating GCs (Starkman et al., 1992; McEwen, 1999; Pruessner et al., 2005). One MRI study has shown transient smaller volumes in key cognitive regions (such as the hippocampus) in breast cancer survivors treated with chemotherapy compared to those treated with surgery alone. These structural differences corresponded to lower scores in attention, visual memory, and concentration 1 year after chemotherapy (Inagaki et al., 2007).

It is not only the effect of GCs on structures like the hippocampus that may play a role in CRCI. The presence of higher circulating GCs expected during cancer diagnosis and treatment may also contribute to an increase in capillary permeability of the blood brain barrier (Tuxen and Werner, 1994) that could lead to augmented accessibility of chemotherapy agents to the brain. Contrary to previous thinking, antineoplastic agents (widely used in chemotherapy) appear to be able to pass through the blood brain barrier to penetrate the brain. This has been demonstrated in studies revealing higher than expected concentrations of antineoplastic agents in both brain tissue and cerebrospinal fluid (CSF) of treated patients (Troy et al., 2000; Tuxen and Werner, 1994). Therefore, increased GC levels could potentially exacerbate the adverse effects of chemotherapy on the brain.

To date, a "flattened cortisol secretion pattern" has been reported in patient populations with breast cancer (Abercrombie et al., 2004; Spiegel et al., 2006). Such a flattened pattern of cortisol secretion has been associated with impaired negative feedback of the HPA axis (Kumari et al., 2010). Meanwhile, significant group differences concerning diurnal cortisol reactivity between chemotherapy-treated patients and healthy controls are not always revealed (Kesler et al., 2009). Future research should sample diurnal cortisol and other distress variables in a larger sample, additionally controlling for other confounding variables such as surgery and type of chemotherapy regimens. Overall, given the possible role of GCs in cognitive disturbances related to cancer and cancer treatment, quantifiable measures of stress should be considered in future studies of CRCI.

3.1.2. Hemoglobin and fatigue

Another factor postulated to underlie fatigue and cognitive symptoms in cancer patients is chemotherapy-induced anaemia, although empirical evidence on this relationship has been conflicting (Ahles and Saykin, 2001; Bender et al., 2006; Castellon et al., 2004; Chang et al., 2004; Jacobsen et al., 2004; Jenkins et al., 2006; Mar Fan et al., 2005; Massa et al., 2006; O'Shaughnessy et al., 2005; O'Shaughnessy, 2006; Schagen et al., 1999; Servaes et al., 2002; Servaes et al., 2005; Shilling et al., 2001; Tchen et al., 2003; van Dam et al., 1998). While it is usually assumed that cognitive disturbance is secondary to fatigue, it could also be the case that an increase in neural effort required to complete day to day tasks gives rise to fatigue. fMRI may yield some objective evidence on this issue, provided that good measures of fatigue and anaemia are included in functional neuroimaging studies of CRCI.

3.1.3. Estrogen

It has also been suggested that chemotherapy might affect cognition by lowering estrogen levels. Treatment-induced menopause has been identified as a risk factor for development of cognitive deficits in breast cancer patients (Jenkins et al., 2006), and the deficits reported by chemotherapy-treated breast cancer patients are similar to those reported after natural or surgical menopause (Bender et al., 2001). The brain is rich in estrogen receptors, including areas involved in memory and executive functioning (O'Shaughnessy et al., 2005). Estrogen has beneficial effects on cognitive function as it helps in neurogenesis, promotes synaptogenesis in the hippocampus CA1 region, and has a role in neuroprotection (Ahles and Saykin, 2002; Henderson, 1997; McEwen and Alves, 1999; Sherwin, 1998). Decreased estrogen levels in healthy post-menopausal women have been associated with poorer verbal learning and memory (Ahles and Saykin, 2002; Shilling et al., 2001). Previous research suggests a "neuroprotective" role of estrogen in key memory structures such as the hippocampus (Lord et al., 2008). Growing recognition of the effects of estrogen on cognition has led to examination of the cognitive impact of adjuvant hormonal therapies for breast cancer. In cases where the breast tumour expresses estrogen and/or progesterone receptors, such hormonal therapies are administered for long periods of time (five years or longer). These therapies act either by selectively blocking estrogen receptors (selective estrogen receptor modulators, or SERMS, such as tamoxifen) or by prohibiting estrogen synthesis (aromatase inhibitors such as arimidex). Some studies have shown that breast cancer patients who received anti-estrogen therapy (in particular in tamoxifen-users) have increased memory problems, (Paganini-Hill and Clark, 2000; Palmer et al., 2008), but others find no such relationship

(Hermelink et al., 2008; Jenkins et al., 2008; Marriott et al., 2004). While estrogen and cognition is extensively studied in an aging population and is applicable in a mostly older cancer patient group (Dumitriu et al., 2010; ; Norbury et al., 2008; Silvia et al., 2001; Thakur and Sharma, 2006), more research focused on estrogen and cognition particularly in the chemotherapy-treated population is needed. (For more information, see Schilder and Schagen (2007) for a review on the cognitive effects of hormonal therapy in breast cancer patients.)

3.1.4. Cytokines

Changes in cytokine activity related to cancer and cancer treatment is another potential mechanism for CRCI as such changes can result in fatigue and cognitive dysfunction. Cytokines are any number of small proteins that are secreted by the immune system and that interact with and modulate the activity of other cells in the immune system (i.e., they are immunomodulatory proteins). Overproduction or inappropriate production of certain cytokines can result in disease, inflammation and tissue destruction. Cytokine activity in the hippocampus has been linked to a disruption in memory function, in particular memory consolidation (Maier and Watkins, 2003). Ongoing studies have reported that levels of multiple cytokines are elevated in all cancer patients, not just in chemotherapy-treated patients. These values were highest post-surgery and remained higher than controls for 6-60 months after diagnosis 2(Vardy et al., 2007). The role of elevated cytokine levels in cognitive impairment should be investigated further in future studies.

3.1.5. Cancer itself

A major weakness of the majority of previous studies of chemotherapy-related cognitive dysfunction is the inclusion of data from post-chemotherapy patients only. A baseline assessment of a patient before treatment is essential in order to demonstrate that any changes in cognition are related to exposure to chemotherapy. More recent studies of CRCI have adopted this prospective approach and report cognitive deficiencies in cancer patients even before exposure to adjuvant treatment. Cimprich et al. (2010) revealed pre-treatment cognitive impairments in 10 newly-diagnosed female breast cancer patients and 9 healthy controls (women with a negative mammogram in the last year). Participants completed a modified Verbal Working Memory task (VMT) during fMRI in order to examine selective attention and working memory. BC patients were less accurate and slower than controls in the high-demand condition of the VMT task. They also showed larger activation than controls in the right inferior frontal gyrus with more cognitive demand, as well as additional components of attention/working memory circuitry in both hemispheres. This study is pivotal in highlighting the importance of baseline assessments of cancer patients before treatment. Without appropriate baseline assessments and scans, the effects of the disease itself could be mistaken for an effect of treatment. This study (Cimprich et al., 2010) appropriately suggests that stress of a new cancer diagnosis, fatigue and potential sleep loss may also contribute to pre-treatment cognitive problems.

3.2. Appropriate controls required

Recruitment of chemotherapy-treated cancer patients in neuropsychological and imaging studies can be quite challenging and costly. Therefore, chemotherapy-treated study groups tend to be relatively small. However, attention must be paid to the fact that

most of the studies examining chemotherapy-treated cancer patients occur in racially and ethnically homogeneous samples, which means that results from these studies may not be truly generalizable. Since controls are also matched to these patients, having the same background, a much larger and varied sample is required.

A control group matched to the patient population in question is essential for appropriate interpretation of study results. CRCI studies are beginning to adopt a prospective design by following the patient group before, during and after chemotherapy. In order to accurately control for potential practice effects on tasks over time, one must have a matched control group. In CRCI research, there is considerable debate on the best "control" population with the ideal as a "control" population that only differs on chemotherapy treatment. Most studies adopt a "healthy", matched control group, as it is the easiest to form considering how difficult it is to match appropriate controls with chemotherapy-treated patients. However, using a "healthy" control group can present a few procedural difficulties. Although these patients have similar age, sex and education profiles, these "healthy" participants fail to match on many other variables. These groups will have different cancer-related cytokine activity, stressors related to cancer diagnosis and treatment, side effects of triggered early menopause and even potential effects of anesthesia from surgery.

Other studies have attempted to control for the "presence" of cancer by not only including a "healthy" control group, but also a cancer group receiving radiation. Participants in the latter group experience most of the same "cancer-related" biological and psychological effects, making the groups more homogeneous, but radiation effects themselves must be considered and how comparable these radiation patients are to the

chemotherapy patients after treatment requires further investigation. Research has shown that fatigue is the major symptom associated with radiation treatment in breast cancer patients (Noal et al., 2010) and fatigue is a confound factor which undeniably affects task performance. Further research into the differences between these 2 types of treatment in cancer patients outside of brain radiation and their potential differential effects on cognition is required. Therefore, admittedly these patients have the same cancer profile (stress, surgery, etc…) but more research is needed to truly identify if their different treatment may be a confound itself. Furthermore, previous studies have indicated that matching cancer patients (chemotherapy-treated and cancer controls) along one treatment is a taxing recruitment scenario. Yet, the reality for many cancer patients is the application of multiple treatment regimens, (ex: surgery + chemotherapy + radiation or additional hormonal treatments) which the field has barely started to investigate. It is difficult to understand the impact of one treatment alone and thus multiple treatment regimens are even more challenging and present an even larger recruitment issue for appropriate controls.

Finally, another interesting control group, used in the Inagaki (2007) study, is a cancer patient population who only underwent surgery, and no other therapies. This is a challenging group to recruit since a majority of patients who undergo cancer-related surgery will subsequently undergo another treatment (chemotherapy, radiation, hormonal). Yet, such a group could help tease out possible confounding variables existing before chemotherapy treatment begins, especially those related to anesthesia-related cognitive deficits. Previous literature has indicated that there may be cognitive decline after general anesthesia administration during surgery. One study (Monk et al.,

2008) examined postoperative cognitive dysfunction in 1064 major non-cardiac surgery patients. They revealed that such deficits are common in adult populations of all ages, but in particular in the elderly (age 60 or older) who are additionally more likely to develop long term deficits. Therefore, there are grounds to acknowledge that anesthesia administration has cognitive effects that could be misattributed to CRCI deficits in the future, especially since the majority of cancer patients are middle aged to elderly populations. However, while this population can help tease out effects such as stress of diagnosis and effects of anesthesia, finding such patients who additionally "match" the patients in the chemotherapy group would be very difficult.

Clearly, one of the major challenges in CRCI research is the choice of an appropriate control group. Recruiting healthy participants is an solid choice since it is already very difficult to find appropriate controls matched to chemotherapy-treated patients on many factors (for example: age, education, sex) and to find individuals in this increasingly aging population who do not have their own health issues (for example: history of mental illness, history of cancer, heart disease, etc…). Some studies (Saykin et al., 2003) have included not just one healthy control group but have additionally included a local-therapy group (surgery and radiation). Matching two different controls per chemotherapy-treated participant does create a stronger study. However, to really identify any effects would require a much larger population than the one presented in the above study.

3.3. Consistency in fMRI results required

Currently, the interpretation of findings in CRCI studies, as well as the translation of results to clinical practice is restricted since there is still limited research on this topic

and a lack of test-retest evidence. Anatomical studies do show some overlap in the regions reported as being significantly different in cancer patients after chemotherapy treatment and matched controls. In particular, bilateral frontal regions are most affected in the post-treatment patient population, which corresponds to the executive functioning deficits (such as problems with planning and working memory) that are consistently reported by patients. Therefore, it is no surprise that functional imaging investigations have largely focused on investigating an important component of executive functioning – working memory. Working memory tasks are geared to largely recruit frontal regions, along with associated temporal and parietal regions- functional studies of CRCI consistently show neural activation differences between chemotherapy-treated patients and controls in these regions. However, the direction of this activity is varied since patients in some studies reveal increased activations while activity is lessened in other studies. This variability in activations related to memory is due to many factors. First, while many of these tasks measure working memory, not a single study has used the same task with the same parameters. Even a subtle variation within an fMRI task can cause significant differences on the overall study results (example: auditory versus visual task stimuli, verbal versus motor responses, longer versus shorter task periods, longer versus shorter rest periods, nature of the control condition, etc…). Therefore, although these tasks all measure some components within the working memory umbrella, they are essentially different from each other in many facets making it difficult to compare and generalize the results. Second, each study uses a very particular and different control group(s), varying from another patient population to healthy controls, as discussed above. Third, there is variability existing within the patient population itself, related to already-

discussed factors such as days since surgery for the baseline scan or the time span between end of treatment and scan dates in cross-sectional studies. In order to better understand working memory and possible confounding variables in CRCI, future studies should adopt similar control groups and use comparable imaging tasks (for example: N-back working memory task), while minimizing variability within the patient population on other factors unrelated to chemotherapy-treatment itself. Additionally, investigations of other executive functioning components, such as response inhibition, that are known to affect working memory should be carefully examined. Overall, the "test-retest" is an important component in any clinical population in order to compare and/or confirm existing results. Such is currently lacking in CRCI imaging research, making it challenging to bridge current experimental data to the future possibilities of clinical practice.

4. Conclusion

The cognitive deficits related to CRCI can persist for years and seriously affect the quality of life of the affected individual, as well as their family and friends. In light of the increasing survival rates for cancer patients, it is essential for patients and their health care providers to understand the long-term adverse effects of therapy.

While neuropsychological assessments indicate that there are true impairments in chemotherapy-treated patients, the recent emergence of neuroimaging in this field has revealed more tangible evidence of changes and differences in the brain. Knowing that the chemo fog phenomenon is not unidimensional, a multifaceted approach is essential if we are to achieve a thorough understanding of the cognitive implications of cancer and cancer treatment. Such studies involve: a prospective design, carefully-chosen and

doubled control groups (ex: healthy controls as well as cancer patients undergoing radiation treatment), dose-dependent neuropsychological testing, multimodal neuroimaging (ex: EEG, anatomical MRI and fMRI assessments), and biological markers sampling (ex: haemoglobin, cortisol, estrogen). Such multifactorial and multimodal studies are most likely to clarify the validity, severity, prevalence, nature, and underlying mechanisms of chemotherapy-related cognitive impairments. Such information should lead to improved support for afflicted patients, including the development of targeted medical and behavioural therapies.

Conflict of Interest disclaimer:

The authors declare that they have no conflict of interest.

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Table 1.1 showing increased (↑) and decreased (↓) volume and/or tissue integrity in chemotherapy-treated patients compared to matched controls from papers reporting detailed regional differences.

Study	Tool	GM and/or WM	Time post-chemotherapy	Frontal and midbrain	Temporal	Parietal	Occipital	Cerebellum
Inagaki et al., 2007	VBM	GM and WM	1 year	GM ↓ R prefrontal cortex WM ↓ B middle frontal gyri, R cingulate gyrus	GM ↓ R parahippocampus WM ↓ L parahippocampus	 WM ↓ L precuneus		
			3 years	None	None	None		
McDonald et al., 2010	VBM	GM	1 month	↓ B middle frontal gyri				↓ L cerebellum
Abraham et al., 2008	FA	WM	22 months	↓ genu of corpus collosum				
Deprez et al., 2010	FA	WM		↓ tissue integrity	↓ tissue integrity			
	MD	WM		↓ tissue integrity				

Table 1.2 showing increased (↑) and decreased (↓) volume and/or tissue integrity in chemotherapy-treated patients over time from papers reporting detailed regional differences.

Study	Tool	GM and/or WM	Time post-chemotherapy	Frontal and midbrain	Temporal	Parietal	Occipital	Cerebellum
McDonald et al., 2010	VBM	GM	1 month vs. baseline	↓ L middle frontal gyrus, B superior frontal gyrus, R medial frontal gyrus, R precentral gyrus , R thalamus, L parahippocampus,	↓ R superior temporal gyrus			↓ B cerebellum
			persisting at 1 year	↓ L middle frontal gyrus, R superior frontal gyrus, R medial frontal gyrus, R precentral gyrus R thalamus				↓ B cerebellum

[a] Note: the nature of the control group varies in each study, please see text body for more details. Abbreviations: GM = grey matter, WM = white matter, L = left, R = right, B = bilateral, Br.= Brodmann area.

Table 2. showing increased (↑) and decreased (↓) activity in chemotherapy-treated patients compared to matched controls during PET and fMRI investigations.

Study	Tool	Tasks	Frontal and midbrain	Temporal	Parietal	Occipital	Cerebellum
Silverman et al., 2007	[O-15] PET	Delayed-recall word memory task	↑ L inferior frontal gyrus (near Br 44 & 45), R superior frontal gyrus		↓L Br 45 (lateral to precuneus), supramarginal gyrus	↓ R primary visual cortex	↑ R posterior lobe
	[F-18] FDG-PET	None (resting metabolism)	↓ L inferior gyrus				
Ferguson et al., 2007	fMRI	Verbal N-back (working memory)	↑ B & widespread		↑ B & widespread		
Saykin et al., 2006	fMRI	Auditory N-back (working memory)	↑ B posterior frontal gyri ↓ B anterior frontal gyri		↑ B posterior parietal lobules		
Kesler et al., 2009	fMRI	Verbal declarative memory- encoding	↓ L superior frontal gyrus, R superior frontal gyrus, B middle frontal gyri		↓ L postcentral gyrus		
		Verbal declarative memory- recall	↑ B basal ganglia, R precentral gyrus, R superior and middle frontal gyri, B inferior frontal gyri, R cingulate gyrus, B insula	↑ R superior temporal gyrus, B fusiform, L hippocampus, B parahippocampus	↑ B precuneus, B superior parietal lobule	↑ B lingual gyri, B cuneus	↑ B cerebellum
de Ruiter et al., 2010	fMRI	Tower of London (planning)	↓ L dorsolateral prefrontal cortex		↓ B posterior parietal lobule		
		Paired-associates task (working memory)	↓ R dorsal striatum	↓ R parahippocampus, L middle temporal gyrus	↓ B lateral posterior parietal lobule, L precuneus, R inferior parietal lobule		

[b] Note: the nature of the control group varies in each study, please see text body for more details. Abbreviations: L = left, R = right, B = bilateral, Br.= Brodmann area

Chapter 3

Title: Structural brain differences in breast cancer patients compared to matched controls prior to chemotherapy.

Short Title: The pre-chemotherapy breast cancer brain

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Cognitive impairment, magnetic resonance imaging (MRI), voxel-based morphometry (VBM), pre-treatment effects, chemofog, chemobrain, surgery, neuropsychology, anatomy.

Abstract

A side effect of chemotherapy can be cognitive impairment. Understanding this relationship requires information on pre-treatment variability between cancer patients and well matched controls. There is a lack of this type of research in the literature. This paper investigated neuroanatomical differences between breast cancer (BC) patients and controls, covarying for possible confounding variables, prior to chemotherapy treatment. Twenty-three female early-stage BC patients underwent MRI scanning after surgery, but before chemotherapy, and were sex-, age- and education-matched to non-cancer controls. MRI images were preprocessed for optimal voxel-based morphometry. Whole brain and region of interest (ROI) group comparisons of grey (GM) and white matter (WM) were performed. Data from neuropsychological tests, hospital records and salivary biomarkers were also analysed. No significant group differences were revealed on neuropsychological tests, estrogen or cortisol levels. Significant ROI structural

differences between BC patients and controls were found with decreased WM in patients in widespread frontal, parietal and temporal regions, as well as insula, striatum and corpus callosum. Interestingly, covarying for factors like “days since surgery”, depression and anxiety revealed increased patient WM compared to controls. GM and WM regressions concerning confounding variables revealed increased heterogeneity of patients compared to controls. This is one of the first imaging studies to focus on pre-chemotherapy neuroanatomical differences between BC patients and well-matched controls, considering demographic, psychological and biological factors in the analyses. Results show structural pre-treatment group differences, highlighting the importance of better understanding confounding factors before considering post-treatment effects.

Introduction

Recent medical advances have greatly improved the odds of achieving long term survival in cancer patients. This has led to increased attention for improving post-treatment quality of life. One factor of particular interest is related to patient self-reports of cognitive impairments following chemotherapy treatment, most often revealed in breast cancer (BC) populations. Patients have coined terms like "chemo fog" and "chemo brain" to refer to these changes that can manifest both during and after treatment. The incidence of chemotherapy-related impairments is highly variable with a range of 17-75% [Correa and Ahles, 1997] reported across neuropsychological studies. Despite this variability, these assessments, along with both structural and functional neuroimaging investigations, support the existence of the self-reported deficits [Abraham et al., 2008; Ahles et al., 1996; Ahles et al., 2002; Bender et al., 2006; Berglund et al., 1991; Brezden et al., 2000; Brown et al., 1998; Castellon et al., 2004; Deprez et al., 2003; de Ruiter et al., 2010; Ferguson et al., 2007; Gottschalk et al., 2003; Hurria et al., 2006; Inagaki et al., 2007; Jenkins et al., 2006; Kesler et al., 2009; Kreukels et al., 2005; Kreukels et al., 2006; Kreukels et al., 2008; Mar Fan et al., 2005; McDonald et al., 2010; Saykin et al., 2003; Saykin et al., 2006; Schagen et al., 1999; Scherwath et al., 2006; Servaes et al., 2002; Shilling et al., 2005; Silverman et al., 2007; Stewart et al., 2007; Tchen et al., 2003; van Dam et al, 1998; Wagner et al., 2006; Wieneke et al., 1995; Wefel et al., 2004], particularly in domains such as working memory, executive function, and processing speed. Previous research has investigated neuroanatomical changes occurring in the chemotherapy-treated brain, which could possibly be related to the reported functional cognitive changes. However, these studies have mostly focused on post-treatment structural changes, without thorough investigations of potential pre-treatment differences between cancer patients and matched controls.

Post-treatment imaging studies have revealed structural differences between chemotherapy-treated patients and controls. In particular, antineoplastic agents (widely used in chemotherapy) are able to pass through the blood brain barrier, penetrate the brain and affect its structure. Previous research has found higher than expected concentrations of such agents in brain tissue and cerebrospinal fluid (CSF) of treated patients [Troy et al., 2004; Tuxen and Werner, 1994]. White matter (WM) is particularly

vulnerable to such neurotoxins and use of MRI techniques has increased recognition of WM changes related to drug intake (ex: leukoencephalopathy in leukemia patients after taking immunosuppressive drugs).

The first study to consider post-chemotherapy structural changes investigated WM volumes in eight advanced stage BC patients receiving high-dose regimens [Brown et al., 1998]. No baseline abnormalities were revealed, yet three out of the four remaining patients in the study showed a progressive increase in abnormal WM volume at three months post-chemotherapy (maximal volume changes ranging from 73-166 cm^3). These volume changes stabilized from 6 to 12 months after chemotherapy completion. These treatment-related WM changes after chemotherapy were not accompanied by persistent neurologic symptoms.

Fluctuations of WM volume is strongly correlated with grey matter (GM) alterations. Voxel-based morphometry (VBM) techniques were applied [Saykin et al., 2003] to investigate whole brain changes in WM and GM in 12 chemotherapy-treated cancer survivors at 5 years post-treatment as well as 12 healthy matched controls. Chemotherapy-treated patients revealed diffuse cortical and subcortical WM volume reductions as well as local bilateral volume reduction of neocortical GM compared to controls.

VBM techniques were also applied to examine brain volume differences between a large sample of BC patients and controls [Inagaki et al., 2007]. Scanning at year one post-treatment (range: 3-15 months) took place with 51 adjuvant chemotherapy-treated BC survivors and 55 surgery-only BC survivors. At year three (range: 27-39 months), there were 73 chemotherapy-treated BC survivors and 59 local-therapy only BC survivors. At the year one testing, chemotherapy patients showed GM losses in the right prefrontal cortex and para-hippocampal gyrus and WM decreases in bilateral middle frontal gyri, left para-hippocampal gyrus, left precuneus and right cingulate gyrus. A strong positive correlation between volume loss and performance on tests of attention and memory was also revealed. Interestingly, these results were no longer evident at the three-year post-treatment scan.

The most recent VBM investigation was the first to apply a prospective design in a moderately large sample and the authors attempted to control for the effects of cancer itself [McDonald et al., 2005]. Seventeen chemotherapy-treated BC patients, 12 non-chemotherapy treated BC patients and 18 healthy controls were scanned at baseline, one month after chemotherapy completion and one year later. Whole brain assessments of GM revealed no baseline group differences, yet a post-treatment decline in both cancer groups compared to controls was revealed. Chemotherapy patients revealed GM reductions in bilateral middle frontal gyri and left cerebellum, while non-chemotherapy patients had decreases in right cerebellum. Also, chemotherapy patients revealed post-treatment decreases in bilateral frontal, temporal and cerebellar regions as well as right thalamus. Meanwhile at one year compared to one month post-treatment, the same patients showed recovery in bilateral superior frontal, left middle frontal, right superior temporal and cerebellar regions, but persistent decreases in right thalamus, medial temporal lobe, left middle frontal gyrus, right precentral gyrus, medial frontal, superior frontal gyri and bilaterally in other cerebellar regions.

Abraham et al. [2008] applied a fractional anisotropy (FA) technique to investigate the WM integrity in the genu and splenium of the corpus callosum in 10 chemotherapy-treated BC patients and nine controls. All 10 patients had self-reported cognitive deficits (avg 22 months post-chemotherapy). In addition to MRI, each participant completed a digit symbol test that showed patients to have slower processing speed than controls. Also, chemotherapy-treatment correlated with lower FA scores in the genu, and lower FA scores correlated with cognitive deficits reported by chemotherapy-treated BC patients.

A more recent diffusion study used both FA and mean diffusivity (MD) techniques to examine cerebral WM integrity in 17 post-chemotherapy BC patients, 10 non-chemotherapy BC patients and 18 healthy controls [Deprez et al., 2010]. Chemotherapy patients showed decreased FA in both frontal and temporal tracts and increased MD in frontal WM compared to both non-chemotherapy patients and controls. These FA scores in the temporal and parietal tracts correlated with neuropsychological tests (attention and processing speed), while self-report cognitive failure questionnaire scores negatively correlated with frontal and parietal WM. Overall, chemotherapy

patients performed worse on neuropsychological tests in contrast to the other groups, with 7 out of 17 classified as clinically impaired. These "impaired" patients had even lower FA values than their "unimpaired" chemotherapy counterparts and controls.

Use of the term chemo fog, as well as the current research focus on post-chemotherapy populations, leads to an assumption that chemotherapy is the sole causative agent of these cognitive impairments. However, one fMRI study examined pre-treatment data and revealed cognitive and neural differences in 10 BC patients compared to 9 healthy controls [Cimprich et al., 2010]. Patients were less accurate and slower than controls in the high-demand task conditions, additionally showing larger activations in right inferior frontal gyrus and bilateral attention/working memory circuitry. This study highlights the importance of baseline assessments since, without an appropriate baseline investigation, the effects of the disease itself or associated confounds could be mis-attributed to an effect of treatment. Several confounding variables may manifest at baseline, and these are now beginning to be considered.

First, some studies have attempted to control for the presence of cancer by including a matched cancer group (not treated with a chemotherapy regimen) [de Ruiter et al., 2010; McDonald et al., 2010; Saykin et al., 2006]. A cancer control may be important since ongoing studies have reported elevated levels of multiple cytokines in cancer patients, with values being highest post-surgery and persisting for 6-60 months after diagnosis [Vardy et al., 2007]. An overproduction or inappropriate production of cytokines can result in disease, inflammation and/or tissue destruction. For example, cytokine activity in the hippocampus has been linked to a disruption in memory consolidation [Maier and Watkins, 2003]. Yet, side-effects of a differing treatment received by non-chemotherapy patients becomes a confound itself. These "other" cancer patients may receive treatments such as radiation or anti-estrogen regimens, both which may contribute to fatigue [Noal et al., 2010] or have also been implicated in cognitive dysfunction [Castellon et al., 2004].

Second, previous literature has suggested a cognitive decline after general anesthesia at all ages, but more commonly in an elderly population (60+) who are more inclined to develop long term deficits [Dijkstra et al., 1999; Dodds and Allison, 1998;

Monk et al., 2008]. Therefore, anesthesia administration during surgery has cognitive effects that could be misattributed to future chemo fog deficits, especially since the majority of BC patients are middle aged to elderly. Possible negative effects of surgery in BC patients have only been investigated in one imaging study [McDonald et al., 2010] with consideration of days since surgery as a covariate. This covariate modified the statistical model, but the results remained unchanged concerning GM volume. However, this study compared two BC groups who had both undergone surgery, thereby both possibly showing the same profile of elevated cytokine levels and cognitive deficits related to general anesthesia. More research is necessary to confirm this result and should be conducted in a larger population.

Finally, undoubtedly a cancer diagnosis gives rise to intense chronic stress for many individuals. Stress, routinely measured in chemo fog studies through self-report, correlates highly with complaints of cognitive disturbances [Bender et al., 2006; Castellon et al., 2006; Jenkins et al., 2006; Schagen et al., 1999; Schagen et al., 2002; Shilling and Jenkins, 2007; Shilling et al., 2005; van Dam et al., 1998]. Cortisol is the stress hormone in humans, and elevated levels are related to increased activity of the hypothalamic-pituitary-adrenal (HPA) axis and/or administration of therapeutic glucocorticoids (part of the immunosuppressive therapy used in cancer treatment). Elevated cortisol levels have been shown to affect brain anatomy [Bremner et al., 1999; McEwen and Magarinos, 1997; Sapolsky, 1985] as well as memory functions [Lupien et al., 1999]. One particularly vulnerable region in the brain is the hippocampus, with smaller volumes seen in populations with higher stress reactivity [McEwen, 1999; Pruessner et al., 2005; Starkman et al., 1992] and transiently in chemotherapy-treated BC survivors [Inagaki et al., 2007]. Cortisol may indirectly play a role in the development of post-chemotherapy cognitive deficits since elevated levels during stress (ex: a cancer diagnosis and/or treatment) have been shown to augment capillary permeability of the blood brain barrier [Tuxen and Werner, 1994]. This increased permeability means that cortisol could potentially exacerbate the adverse effects of chemotherapy agents in the brain.

Finally, considering the typically older BC population as well as their menopausal state, measuring baseline estrogen level is essential. Estrogen has been suggested to have

neuroprotective qualities, thereby helping maintain cognitive capacities, but the exact mechanisms by which it exerts such protection remains unclear. Previous research has suggested an interaction between various neurotransmitter systems, neurotrophic factors, as well as nuclear estrogen receptors. The latter are particularly present in areas such as the amygdala and hippocampus, both involved in memory and learning (Behl 2002; McEwen, 2002; Shughrue and Merchenthaler, 2000). Post-menopausal studies have indicated that women report declining cognitive capacities and show decreased bilateral hippocampal volumes. Interestingly, this effect has been shown to be minimized by estrogen therapy [Lord et al., 2008].

Overall, there are few neuroimaging investigations of cancer patients prior to commencement of chemotherapy-treatment. Results from existing research are conflicting and thus, further pre-chemotherapy research is required. The current paper investigated baseline neuroanatomical differences in new BC patients (prior to chemotherapy) compared to individually-matched healthy controls. Suggested baseline confounds associated with cancer populations were considered as covariates, including: days since surgery, diurnal cortisol levels, estrogen, depression and anxiety inventories, and IQ. It was hypothesized that there would be both grey and white matter volumetric differences between patients and controls at baseline. It was also hypothesized that consideration of suspected confound variables may modify the analyses with no covariates (as seen in previous studies [McDonald, 2010]), reveal additional volumetric group differences and/or expose the highly heterogeneous nature of the patient population. Volumes were assessed by whole brain analyses and by ROI investigation of brain areas reported in the post-chemotherapy literature.

Methods

Twenty-three newly diagnosed breast cancer (BC) women were recruited through the Ottawa Hospital Regional Cancer Centre (average age: 51 ± 8.5 years, range: 35-64). At the time of the MRI scan, patients had undergone mastectomy or lumpectomy for early stage BC and were scheduled to commence a chemotherapy regimen. Inclusion criteria were: female, no previous cancer or cancer treatment, no unstable psychiatric/ neurological illness, no history of substance abuse, Beck Depression Inventory (BDI-II;

[Beck et al., 1996]) scores of < 20 and < 15 for the Beck Anxiety Inventory (BAI; [Beck and Steer, 1990]), fluency in English, and minimum grade 8 education. MRI exclusion criteria included left-handedness, sight problems, claustrophobia, pacemaker, and metal implants. Healthy controls (average age: 49 ± 9 years, range: 30-62) were recruited by a combination of patient nominations and through posters/website ads. Each non-cancer control was sex-, age- and education-matched to each individual cancer patient and also met the above inclusion and exclusion criteria. Controls followed the same procedure as their index BC patient.

Neuropsychological Assessments:

All participants completed baseline psychometric tests as well as an MRI scanning session. The 2 ½ hour baseline psychometric battery was comprised of: social / medical history, classic pencil-and-paper tests, and a 30 minute computerized cognitive test (CNS Vital Signs; https://www.cnsvs.com/). This computerized test battery has been validated [Gualtieri and Johnson, 2006] in a broad age range and across clinical and non-clinical populations (including cancer patients), as well as proven sensitive in monitoring patient's cognitive status over time. In particular, it measures: attention, reaction time, working memory, executive function as well as visual and verbal episodic memory. Raw neuropsychological data were converted to standardized scores based on the means and standard deviations of the matched healthy control group. Summary scores for several cognitive domains were computed as well as an overall cognitive summary calculated by averaging all of the cognitive scores. Cognitive domains were computed based on both rational and empirical grounds. Nineteen tests comprised the neuropsychological battery from which 6 cognitive domains were extracted: 1. Verbal Memory (Hopkins Verbal Learning Test-Revised (HVLT-R; [Brandt and Benedict, 2001])) and CNSVS Verbal Memory), 2. Processing Speed (WAIS-III [Wechsler, 1997] Digit-symbol coding, WAIS-III Symbol Search, controlled oral word association [Benton et al., 1994], CNSVS Processing speed, CNSVS Executive Functioning, CNSVS Reaction time and CNSVS Cognitive Flexibility), 3. Attention (WAIS-III Digit Span, WAIS-III Letter-number sequencing, Paced Auditory Serial Attention Test (PASAT; [Gronwall, 1977]), CNSVS Sustained attention and Trailmaking Test, Part B [Army Individual Test Battery, 1944]),

4. Visual Memory (CNSVS Visual Memory, Brief Visuospatial Memory Test (BVMT; BVMT-R; [Benedict, 1997]) and CNSVS Working Memory), 5. Reasoning (CNSVS Reasoning) and 6. Verbal Short-term Memory (Auditory Consonant Trigrams; [Boone et al., 1990]). Additionally, each participant completed the Rosenberg Self-esteem questionnaire (RSE: [Rosenberg, 1985]) and the Questionnaire for Competence and Control (FKK; [Krampen, 1991]) at the scanning session.

Demographic, clinical neuropsychological and self-esteem data were analyzed by two-tailed independent t-tests using Statistical Package for the Social Sciences version 18.0 program (SPSS v. 18.0, Chicago, IL). Correlational analyses were also conducted to reveal any existing relationship between factors.

Assessment of Biological Markers:

Patient hospital record reviews provided baseline blood work (hemoglobin, white blood cell, neutrophil). Salivary samples were collected to assess free cortisol levels, a valid biological marker of the free fraction of the major human stress hormone [Kirschbaum and Hellhammer, 1994]. At-home sampling kits were given to individual participants, each containing 14 cotton salivettes for cortisol sampling and detailed instructions. Participants were asked to follow a rigid sampling schedule for two typical and consecutive weekdays. Cortisol was sampled each of the two days at: Awakening, 30 min after awakening, 1 hour after awakening (participants could consume food and drink after this sample), 10am, 2pm, 6pm and 9pm. This schedule provided a circadian cortisol profile for each participant. This is an important component since circadian cortisol rhythms are affected in high stress and in clinical populations [Backhaus et al., 2004; MacHale et al., 1998; Monteleone and Maj, 2009; Pruessner et al., 1997; Pruessner et al., 1999]. The completed samples were preserved in a freezer until the next appointment. The saliva samples were analyzed for cortisol using ELISA techniques (according to those described by Salimetrics at salimetrics.com). Intra- and inter-assay variabilities (CVs) for data points (excluding outliers) were less than 10% and 15%, respectively. In order to analyze salivary cortisol differences, the area under the curve from ground (AUCg) for each participant was computed [Pruessner et al., 2003]. AUCg was computed in Excel and 2-tailed independent group t-tests were applied using SPSS v. 18.0.

Pure spit estrogen samples were collected with the same at-home sampling kits presented above. Samples were completed at the 10am mark for each of the 2 consecutive days. The saliva samples were analyzed for estradiol using ELISA techniques (according to the described by Salimetrics at salimetrics.com). Intra-assay coefficients of variation on one estrogen measurement/participant less than 0.43 (CV (%) = 4.03, Range: 0.148 - 19). Group differences in estrogen levels were assessed by a 2-tailed independent t-tests using SPSS v. 18.0.

Scanning Sessions:

Images were acquired using a 1.5 Tesla Siemens Magnetom Symphony MR scanner. Participants were instructed to lie on their back with their head secured in a standard head holder and to stay as still as possible while in the scanner. A gradient echo localizer was acquired and used to prescribe a subsequent 3D FLASH (Fast Low Angle Shot) spoiled gradient sequence (TR/TE 22/9.2ms, flip angle 30º, field of view (FOV) 256x256 mm analyses). T1-weighted images were processed and examined using SPM8 software (Wellcome Department of Imaging Neuroscience Group, London, UK; http://www.fil.ion.ucl.ac.uk/spm) and the VBM8 toolbox (http://dbm.neuro.uni-ena.de/vbm.html), set with default parameters. Images were bias-corrected, tissue classified, and registered using linear (12-parameter affine) and non-linear transformations (warping), within a unified model [Ashburner and Friston, 2005]. Subsequently, analyses were performed on grey matter (GM) and white matter (WM) segmented volumes, which were multiplied by the non-linear components derived from the normalization matrix in order to preserve actual GM and WM values locally (modulated GM and WM volumes). The modulated volumes were smoothed with a Gaussian kernel of 12 mm full width at half maximum (FWHM).

Total WM, GM and CSF volumes were extracted per participant and correlation analyses were computed in SPSS along variables such as: age, IQ and the six neuropsychological cognitive domain scores. Voxel-wise GM and WM group differences between BC patients and matched controls were examined using independent-sample t-tests. In addition, these independent sample t-tests were individually performed with separate covariates (days since surgery, diurnal

cortisol, depression scores, anxiety scores, IQ) in order to isolate the influences of suspected confound variables on group differences. Regression analyses of all participants, patients alone and controls alone were also computed along the same confound variables in order to investigate sample heterogeneity. For both analyses, voxels with GM or WM values of less than 0.1 (absolute threshold masking) were excluded in order to avoid possible edge effects between different tissue types.

Whole brain and ROI investigations were conducted at a set threshold of $p_{uncorr.}$= 0.001 uncorrected, with a cluster-wise correction at p_{FWE} = 0.05 and a set cluster size larger than 10 voxels. ROIs investigated in this study were selected from several sources: the affected regions in previous post-chemotherapy structural and functional studies [Kreukels et al., 2005; Kreukels et al., 2006; Saykin et al., 2006; Silverman et al., 2007; Inagaki et al., 2007; Abraham et al., 2008; Kreukels et al., 2008; Deprez et al., 2010; McDonald et al., 2010; Raffa and Tallarida, 2010; de Ruiter et al., 2010], and related papers considered in light of possible confound variable effects, such as stress and cytokine activity. ROIs extracted included bilateral: frontal lobe, precentral gyrus, cingulate gyrus, parahippocampus, hippocampus, insula, thalamus, basal ganglia, temporal lobe, parietal lobe, precuneus, occipital lobe, brainstem and cerebellum. Significant results presented are both un-adjusted and adjusted for multiple comparisons. A Bonferroni adjustment with 28 comparisons lowers the alpha to 3.57^{-5} with a significant z-value now of 3.97.

Results

Demographic and Clinical Data

Correlations were computed to investigate relationships between demographic data and neuropsychological variables. Age negatively correlated with attention ($r(46) = -0.311$, $p= 0.035$), reasoning ($r(46) = -0.418$, $p= 0.004$), and processing speed ($r(46) = -0.531$, $p< 0.001$) with younger participants scoring overall higher on tests comprising these cognitive domains. The Wechsler test of Adult Reading positively correlated with five out of the six cognitive domains: attention ($r(46) = 0.486$, $p= 0.001$), visual memory ($r(46) = 0.332$, $p= 0.026$), reasoning ($r(46) = 0.295$, $p= 0.049$), verbal short-term memory ($r(46) = 0.459$, $p= 0.002$), as well as processing speed ($r(46) = 0.447$, $p= 0.002$).

Individuals with higher Wechsler scores also had higher scores in these cognitive domains. Additionally, verbal memory positively correlated with reasoning ($r(46) = 0.353$, $p= 0.016$), attention correlated with visual memory ($r(46) = 0.496$, $p= 0.000$), reasoning ($r(46) = 0.596$, $p= 0.000$) as well as processing speed ($r(46) = 0.551$, $p= 0.000$) and visual memory correlated with processing speed ($r(46) = 0.314$, $p= 0.033$). As expected, BDI scores positively correlate with BAI scores ($r(46) = 0.342.$, $p= 0.020$). A negative correlation was revealed between BDI scores and the processing speed domain ($r(46) = -0.368$, $p= 0.012$) while a similar negative correlation was shown between BAI scores and verbal short-term memory ($r(46) = -0.362$, $p= 0.013$).

Table I summarizes demographic and clinical characteristics for the BC and the healthy control groups. Estimated IQ was assessed by the Wechsler Adult Reading Task, and no significant group differences between patients and controls were revealed ($t(44) = -1.312$, $p = 0.197$). No significant differences were revealed between groups considering menopausal status, with half the sample being post-menopausal. The BDI revealed larger mean scores in patients (8.3) compared to controls (3.8) ($t(44) = 2.299$, $p= 0.026$). Similarly, the BAI showed larger scores in patients (7.8) compared to controls (4.1) ($t(44)= 2.090$, $p=0.042$). There were no significant group differences in any of the six cognitive domains or the self-esteem measures.

Biological Measures

Patient blood work revealed average values in the normal range: Hemoglobin (HGB): 134 grams/L, White blood cells (WBC): 7×10^9/L, Neutrophils: 4.21×10^9/L. Patients and controls did not differ significantly on salivary estrogen levels ($t(25) = 0.835$, $p>0.412$) with average values being 6.07 (StDev 3.89) and 4.98 (StDev 2.89), respectively. Similarly, group differences concerning AUCg values of diurnal salivary cortisol were also non-significant ($t(25) = -1.308$, $p > 0.203$), with average AUCg at 12.7 (StDev 5.41) and 16.1 (StDev 8.68) respectively for patients and controls. Each subject's diurnal cortisol index, reflecting average diurnal cortisol excursion, ranged from 0 to 0.973 ug/dL, mean = 0.144 (StDev 0.159). See Figure 1 graph of diurnal cortisol rhythms for BC patients and matched controls.

Total volume correlations with demographic, neuropsychological domains and cortisol

Mean segmented volumes for GM, WM, CSF and total brain volume were calculated for both patients and controls. Patients' volumes were GM= 529.05 (StDev 46.98), WM= 579.92 (StDev 68.03) and CSF= 203.31 (StDev 29.95), with total brain volume of 1312.28 (StDev 116.27). Volumes in controls were GM= 530.09 (StDev 56.69), WM= 600.51 (StDev 49.46) and CSF= 209.61 (StDev 34.94), with total brain volume of 1340.22 (106.02). Across all participants, there were positive correlations between GM and WM ($r(46)= 0.634$, $p= 0.01$), as well as between brain total volume with GM ($r(46) = 0.835$, $p= 0.01$), WM ($r(46) = 0.894$, $p= 0.01$) and CSF ($r(46) = 0.450$, $p= 0.01$). There were negative correlations between GM and age ($r(46) = -0.413$, $p= 0.01$), WM and BDI scores ($r(46) = -0.308$, $p= 0.05$) as well as CSF and processing speed ($r(46) = -0.368$, $p= 0.05$). A positive correlation was revealed between GM and total neuropsychology scores (collapsed 6 domains) ($r(46) = 0.299$, $p= 0.05$), as well as between CSF and age ($r(46) = 0.336$, $p= 0.05$). Across controls only, there were positive correlations between GM and WM ($r(46) = 0.643$, $p= 0.01$), as well as between total brain volume with GM ($r(46) = 0.864$, $p= 0.01$), WM ($r(46) = 0.848$, $p= 0.01$) and CSF ($r(46) =0.431$, $p= 0.05$). There was a negative correlation between GM and age ($r(46) = -0.537$, $p= 0.01$), yet positive correlations between CSF and age ($r(46) = 0.438$, $p= 0.05$) as well as GM and visual memory domain scores ($r(46) = 0.414$, $p= 0.05$). Across patients only, there were positive correlations between GM and WM ($r(46) = 0.681$, $p= 0.01$), as well as between total brain volume with GM ($r(46) = 0.832$, $p= 0.01$), WM ($r(46) = 0.930$, $p= 0.01$) and CSF ($r(46) = 0.464$, $p= 0.05$). No other significant correlations were revealed.

Anatomical MRI analysis- Grey matter

Between group analyses (GM)

There were no between-group differences in GM volume in both whole brain and ROI analyses when computing independent t-tests. Addition of the covariates did not significantly modify the results.

Regression Analyses (GM)

See Table II for significance, ROI, MNI coordinates, cluster sizes, t values and z values. The table was grouped according to regression analyses with all participants, controls only and patients only along the five confound factors.

All participants (GM)

Regression analysis of the BDI scores computed with all study participants indicated smaller volume in the left cuneus with increasing depression index scores. Larger BAI scores were associated with larger volumes in the right parahippocampus and the right lentiform nucleus. Regression along the Wechsler assessment revealed larger volumes in the left superior frontal gyrus, left middle frontal gyrus, left inferior frontal gyrus, and left middle occipital gyrus.

Controls only (GM)

Regression analysis with controls along BDI scores revealed larger volumes in the left inferior parietal lobule, left inferior temporal gyrus and right middle orbital frontal gyrus with larger scores in this depression index. Similarly, larger BAI scores were associated with larger volumes in the left middle frontal gyrus and right hippocampus. Regression along Wechsler test scores indicated larger volumes in the left superior frontal gyrus, left cuneus, left putamen, as well as right superior occipital gyrus with larger scores on this IQ measure.

BC patients only (GM)

Regression analysis of BC patients only along “days since surgery” revealed increased volume in left and right thalamus with more post-surgery time passed. Meanwhile, decreased volume was reported in the left cuneus with more days between surgery and scan dates. Regression along BDI scores revealed smaller volumes in the right inferior and middle occipital gyri with larger depression scores. Larger BAI scores were associated with significantly larger volumes in the right lentiform nucleus. Regression along Wechsler scores revealed larger volumes in the left supramarginal gyrus, right inferior parietal lobule, and left posterior cerebellar declive.

Anatomical MRI analysis- White matter

Between group analyses (WM)

No significant group differences in whole brain WM values were revealed between patients and controls when computing independent t-tests. See table III for significance, ROI, MNI coordinates, cluster sizes, t values and z values. However, ROI analyses extracted group differences between patients and controls with patients showing smaller WM volumes in bilateral inferior frontal, left pre and postcentral gyri, left insula, left striatum (putamen and caudate), right supramarginal gyrus, left inferior parietal, right middle temporal gyrus, left precuneus and left corpus callosum (Figure 2). To control for any effects of potentially contributing factors, selected covariates were individually added to the model for analyses. Overall, patients revealed larger volumes with the addition of certain covariates such as "days since surgery", BDI scores and BAI scores. Meanwhile, all other significant findings (with or without the addition of a covariate) included smaller WM volumes at baseline in BC patients compared to controls.

Regression Analyses (WM)

See Table IV for significance, ROI, MNI coordinates, cluster sizes, t values and z values. The table was grouped according to regression analyses with all participants, controls only and patients only along the 5 confound factors.

All participants (WM)

Regression analyses of all study participants along cortisol revealed increased volume in the left precuneus and left cuneus. Regression along BDI scores revealed both positive and negative relationships with WM volume. Higher BDI scores were associated with larger volumes in the left inferior temporal gyrus and left superior temporal pole. Meanwhile, higher BDI scores were associated with smaller volumes in the right superior frontal gyrus, left medial frontal gyrus, right inferior frontal operculum, bilateral anterior cingulate gyri, left insula, right caudate, left superior parietal lobule, left precuneus, right posterior cerebellar declive, left posterior cerebellar tonsil and left anterior cerebellar

culmen. Regressions along BAI scores revealed increased left angular gyrus volume and decreased volume in the left inferior orbital frontal gyrus with larger anxiety scores. Higher scores on the Wechsler measure were associated with larger volumes in the right precentral gyrus, right postcentral gyrus, right precuneus and left cuneus.

Controls only (WM)

Regression analysis with controls only along BDI scores revealed decreased volume in the left middle frontal gyrus and left precentral gyrus with increasing scores. Additionally, larger volumes were revealed in the left posterior cerebellar declive with larger BAI scores. Finally, larger volumes were revealed in the right precuneus, right posterior cingulate gyrus, right caudate and left cuneus with larger IQ scores (Wechsler).

BC patients only (WM)

Regression analysis of BC patients only with "days since surgery" revealed increased volume in left insula and putamen with more time passed between surgery and the scanning session. Meanwhile, smaller volumes were reported in the right inferior frontal gyrus with more post-surgery days. Regression along cortisol AUCg showed larger volumes in the right middle and superior frontal gyri, right cingulate gyrus, left rolandic operculum and left anterior cerebellar culmen. Investigations along BDI scores revealed smaller volumes in the right inferior frontal and right brain stem with larger depression scores. Larger BAI scores were associated with significantly larger volumes in the left angular gyrus but smaller volumes in the superior medial frontal gyrus. Regression along IQ scores (Wechsler) revealed an association between smaller right and left corpus callosum volumes and larger IQ scores.

Discussion

Previous research has indicated that chemotherapy-treated cancer patients experience a post-treatment cognitive decline. However, before assessing cognitive deficits precipitated by chemotherapy, one must better understand these patients at baseline. While there are few systemic studies examining distinctions between patients

and matched controls at baseline, these do suggest group differences in brain function and structure even before treatment commences [Cimprich et al., 2008; Vardy et al., 2007]. Such baseline distinctions between newly diagnosed BC patients and matched controls are supported by the current results.

No group differences regarding GM were revealed in this study, yet there were significant differences in WM volume between patients and controls. The latter volumetric difference is interesting, considering a strong focus on WM changes in the study of chemofog. Therefore, some of the WM differences attributed to post-treatment effects may already be manifesting at baseline. It is important to remember that this study applied both whole brain and ROI analyses. While whole brain results were not significant, ROI investigations based on post-chemotherapy findings yielded significant group differences. ROI analyses are appropriate for investigations of subtle group differences in brain volume when previous research indicates target regions that warrant further exploration. Whole brain analyses are extremely stringent and increase type II error since all voxels in the brain are considered. ROI analyses consider a smaller number of voxels contained to a limited masked area, which increases the chance of uncovering subtle group differences. While this technique increases the odds of committing a type I error, this can partially be controlled for with more stringent thresholding. Therefore, other structural MRI studies may not have uncovered baseline group differences due to the fact that their investigations were limited to "whole brain" analyses. The current paper revealed smaller pre-chemotherapy WM volumes in patients compared to controls in widespread regions in the frontal, parietal and limbic regions using ROI analyses.

This paper is also the first and largest baseline structural imaging study to systematically covary a range of suspected confound variables in this patient population. A variety of covariates were examined such as diurnal cortisol, depression and anxiety scores, IQ scores as well as days since surgery. The addition of covariates in the independent t-test did not unveil volumetric group differences concerning GM, but considerably modified WM results. First, when considering days since surgery as a covariate, patients revealed previously unobserved larger WM volumes compared to controls in left middle occipital gyrus and right parahippocampus. Meanwhile, when considering smaller patient WM volumes compared to controls, addition of this particular

covariate eliminated all baseline differences and attenuated the group difference in the left insula. A smaller left temporal pole in the patient cohort was also introduced with this covariate, this region being highly connected with both the limbic system and the insula [Chabardès et al., 2002]. Interestingly, the left temporal pole has also shown decreased glucose metabolic rates with increasing number of chemotherapy cycles [Baudino et al., 2011]. It is possible that anesthesia agents or post-surgery cytokines first impact this region, and these adverse effects are then sustained and enhanced with chemotherapy treatment. Considering that BC patients are usually older, it is also possible that these effects exacerbated volumetric and functional declines seen in this region which also take place with normal aging [Bergfield et al., 2010; Dupont, 2002; Eberling et al., 1995; Goldman-Rakic and Brown, 1981]. Overall, the number of days since surgery impacts WM volume in BC patients.

Abnormal diurnal cortisol patterns have been reported among women with BC [Abercrombie et al., 2004; Spiegel et al., 2006], with patients showing "flattened cortisol rhythms" [Spiegel et al., 2006]. Flatter patterns of cortisol secretion have been associated with impaired negative feedback of the HPA axis [Kumari et al., 2010]. Similarly, a flattened response was observed in patients in the present study, as revealed in a graph investigation of averaged areas under the curve (AUC). However, there was no significant difference in the AUCg between patient and control groups, a result which is supported by another fMRI study [Kesler et al., 2009] who considered "distress measures" such as salivary cortisol in their investigation of verbal declarative memory capacities in patients after treatment. It is important to note that both the Kesler study [2009], as well as the current study examined diurnal salivary cortisol levels in small populations, potentially too small to uncover significant group differences. (Practical constraints in the current study prevented many of the participants from completing salivary sampling.) Yet, in this study, the addition of diurnal cortisol values as a covariate in the t-test analysis only maintained volumetric group differences in the left pre- and post-central gyri seen in the no-covariate condition and eliminated all other group differences. Interestingly, this covariate additionally introduced new WM group differences with smaller patient volumes in the left middle temporal, right superior frontal, right inferior parietal (angular) lobule and right middle occipital gyrus. Therefore,

consideration of cortisol not only modified the no-covariate model, but also introduced new group differences in widespread regions of the brain. Additionally, the standard deviations were rather large in this patient group, especially for the morning sampling time points where the group differences are the largest. Future investigations of baseline cortisol differences between BC patients and controls should be conducted in a larger sample. Patients are in a high stress situation with a new cancer diagnosis, therefore it is not surprising that they are showing group differences compared to controls when cortisol, an indicator of stress, is being considered.

When the BDI and the BAI scores were added in separate group comparisons as covariates, no new regions were introduced compared to the no-covariate condition. However, both inventories attenuated volumetric differences manifesting as smaller WM volumes in patients compared to controls. While there were subtle discrepancies in the regions affected by each inventory, there was considerable overlap given the correlation of these factors. What is interesting is that, while participants as a whole scored in the mild range for both inventories, patients had significantly larger scores compared to controls. Such a divergence between groups could be the consequence of a variety of factors present in the patients' lives such as the stress of a new diagnosis and/or facing disease side-effects including upcoming cancer treatment. Hence, even a subtle but significant difference in these "mild" scores should be investigated at baseline, as this study reveals influences both behaviourally and in the brain.

Finally, the addition of the IQ covariate in the model does not uncover new group differences, yet modifies the no-covariate model. More investigations are necessary before drawing a conclusion on the relationship between baseline factors in cancer patients, IQ and brain volume.

The effects of confound factors which may contribute to the heterogeneity of a population, can be considered in regression analyses. The exact regions that modulate volume along a particular variable are presented in Tables II and IV, but these and their functions will not be further discussed. While within-sample modulations along variables are expected, attention must be drawn to the minimal overlap in regions seen in patient and control groups (23 participants in each group) for each separate regression analysis. Patients showed different volumetric variations along factors compared to controls.

Additionally, new anatomical variations along the factors in question were introduced when performing regressions with all participants (patients and controls together, 46 subjects). Therefore with all participants, new areas were uncovered that were not revealed in single group analyses (patients alone or controls alone). When the sample is doubled, more areas are extracted which highlights the need for larger populations in chemofog studies. Also important to consider, while heterogeneity is to be expected in both patient and control groups alike, the patient group revealed significantly more within-group variability compared to controls along certain confound factors. This is particularly evident in the cortisol AUCg and WM volume regression analysis where patients showed larger volumes associated with larger AUCg in widespread frontal regions and cerebellum, while controls had no within-group variability.

The regression analysis considering "days since surgery" in the patient cohort revealed further within-group variability. With more days between the surgery and scan dates, patients revealed larger left insula and putamen WM volumes as well as bilateral thalamus GM volumes. Meanwhile, with fewer days post-surgery, patients showed smaller right inferior frontal WM volume. Such results are further support for a potential effect of surgery on brain anatomy, possibly due to negative effects of anesthesia [Monk et al., 2008] and/or of cytokine activity [Vardy et al., 2007]. Whether patients are "regaining" anatomical volume or these regional time-dependent increases are compensatory in nature, it remains that days since surgery seems to contribute to variability in the patient sample. This particular finding could help explain some of the variability concerning baseline measures in the recently published prospective studies. If a study recruits patients who underwent baseline MRI scans soon after surgery, there may be volumetric differences compared to a patient sample who was scanned much later after surgery. Additionally, while one may assume that post-surgery patients are "returning" to a baseline that resembles controls, such recuperation could take much longer than time allotted in these studies, which is usually about one month. It is important to note that in some cases, a complete recuperation might be an erroneous assumption as previous studies have indicated long-term cognitive deficits [Monk et al., 2008]. It is interesting to consider that confound variables could already manifest at

baseline, thereby suggesting that some of the post-treatment results could in fact be contaminated by these effects.

In conclusion, this study suggests that investigating only whole brain volumetric differences between controls and BC patients is not sufficient to truly understand this multi-factorial patient population. The differences may be subtle and the results from this paper support the use of rigorous and varied statistical measures to expose these effects. Region of interest investigations helped uncover differences between patients and matched controls, focusing on regions that are consistently reported in post-chemotherapy populations. These group differences were only observed in WM volumes, a prominent focus for post-chemotherapy studies. Hence, there is reason to suspect that some of the adverse effects attributed to chemotherapy are already present at baseline, and these are potentially exacerbated by the treatment. This was also supported by the modifications to results observed when adding covariates to the analyses. These confound variables, particularly post-surgical time to scanning and cortisol measures should be included in future studies as primary variables of interest. Additionally, grouping patients together for between-group comparisons (such as t-test) washed out important subtle variations in the patient population. This paper suggests that regression analyses of patients alone can help uncover small changes/differences within the sample along important variables such as days since surgery. Overall, while each suspected factor in this study was applied as a covariate in a separate t-test or regression analysis, it is acknowledged that these do not present alone and likely work in conjunction. Baseline assessments are important to validate each BC patient's personal experiences. While chemotherapy has been shown to affect cognition and the brain, there are a number of obstacles that patients must face even before the commencement of treatment - whether at the biological level with elevated cytokine activity and the side-effects of surgery, or at the personal level with increased feelings of stress, anxiety and depression. Finally, this paper provides preliminary but compelling evidence of neuroanatomical differences between BC patients and controls, prior to chemotherapy. Such findings are important since volumetric differences/changes in the brain could lead to functional impairments in cognitive processing.

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Table I. **Demographic and Clinical Data**

Factors	Patients (n=23)	Controls (n=23)
Age[a]	51.5 (8.47)	50.4 (8.82)
Estimated IQ[a]	105.8 (9.05)	109.22 (8.43)
Education Level[b]		
High School	2	3
College	10	11
Bachelors	6	3
Masters	3	6
PhD	1	0
Marital Status[b]		
Married / Common Law	19	14
Single	0	3
Separated / Divorced	2	6
Widowed	2	0
Days since surgery[c]	47.6 (Max 71, Min 28)	---
Cancer stage		
1	4	---
2a	10	---
2b	5	---
3a	4	---
Menopausal status[b]		
Menstruating	8	9
Perimenopausal	3	2
Postmenopausal	12	12
BDI *[a]	8.3 (8.92)	3.78 (3.06)
BAI *[a]	7.81 (7.25)	4.13 (4.35)
Neuropsychological Domain scores		
1. Verbal memory	-0.24 (1.19)	---
2. Processing speed	-0.11 (0.91)	---
3. Attention	-0.26 (0.86)	---
4. Visual memory	0.22 (0.78)	---
5. Reasoning	-0.35 (1.48)	---
6. Verbal short-term memory	-0.17 (0.65)	---
Mean segmented brain volumes		
Grey matter	529.05 (Max 640.09, Min 450.40)	530.09 (Max 622.41, Min 438.08)
White matter	579.92 (Max 711.13, Min 454.36)	600.51 (Max 698.83, Min 521.55)
CSF	203.31 (Max 257.82, Min 155.26)	209.61 (Max 298.80, Min 156.18)
Total brain	1312.28 (Max 1492.54, Min 1123.56)	1340.22 (Max 1496.24, Min 1120.18)

* = Significant group difference (2 way independent t-test, $p < 0.05$)
[a] Age, Estimated IQ, BDI, BAI, Domain scores = Mean (StDev).
[b] Educational level, Marital Status, Menopausal status = n.
[c] Days since surgery: # of days between surgery and baseline scan.
Domain scores = standardized to controls.
Abbreviations: IQ= Intelligence quotient, BDI = Beck Depression Inventory, BAI = Beck Anxiety Inventory

Table II. **Significant[a] regression results for grey matter in all participants, patients and controls**

All participants

Condition	Region	Atlas	P_{FWE}	K_E	*T* value	*Z* value
Cortisol (+)	None					
Cortisol (-)	None					
BDI (+)	None					
BDI (-)	L Cuneus (-12 -91 13)	aal	0.045	20	3.44	3.22
BAI (+)	R Parahippocampus (35 -31 27)	TD labels	0.045	82	3.87	3.57
	R GP., lentiform nucleus (17 6 -5)	TD labels	0.018	144	3.85	3.55
BAI (-)	None					
Wechsler (+)	L Inferior frontal gyrus (-45 42 4) L Inferior frontal gyrus (-45 30 9)	TD labels	0.024	442	3.83 3.46	3.83 3.24
	L Middle frontal gyrus (-30 60 6) L Middle frontal gyrus (-42 47 7)	aal	0.016	583 3.68	4.66 3.68	4.18 3.42
	L Superior frontal gyrus (-17 59 33)	aal	0.034	300	4.17	3.81
	L Middle occipital gyrus (-14 -94 12) L Middle occipital gyrus (-18 -102 3)	TD labels	0.038 0.047	152 101	4.01 3.60	3.68 3.35
	L Cerebellar tonsil (-26 -36 -48) L Cerebellar tonsil (-33 -63 -48)	TD labels	0.012 0.040	378 53	4.58 3.61	4.12 3.36
Wechsler (-)	None					

Patients only

Condition	Region	Atlas	P_{FWE}	K_E	*T* value	*Z* value
Days since surgery (+)	L Thalamus (-8 -4 -2)	aal	0.022	57	5.86	4.46
	R Thalamus (6 -6 -3)	aal	0.025	37	4.74	3.87
Days since surgery (-)	L Cuneus (-18 -88 16)	TD labels	0.031	186	5.68	4.37
Cortisol (+)	None					
Cortisol (-)	None					
BDI (+)	None					
BDI (-)	R Inferior occipital gyrus (50 -76 -3)	aal	0.045	68	4.40	3.66
	R Middle occipital gyrus (50 -76 -3)	aal	0.021	97	4.73	3.86
BAI (+)	R GP, lentiform nucleus (15 5 -6)	TD labels	0.031	35	3.90	3.34
BAI (-)	L Precuneus (-3 -72 40)	aal	0.064	81	4.04	3.44
Wechsler (+)	R Inferior obito-frontal gyrus (42 45 -3)	aal	0.045	35	3.93	3.36
	L Inferior frontal tri gyrus (-45 18 27)	aal	0.044	63	3.83	3.30
	L Supramarginal gyrus (-53 -43 33)	aal	0.010	320	4.16	3.88
	R Inferior Parietal lobule (47 -24 28)	TD labels	0.074	21	4.31	3.61
	L Posterior cerebellar declive (-36 -55 -23) L Posterior cerebellar declive (-42 -70 -26)	TD labels	0.009	501	4.19 4.13	3.54 3.49
	L Posterior cerebellar tonsil (-24 -39 -45) L Posterior cerebellar tonsil (-36 -61 -47)	TD labels	0.047 0.051	37 25	3.68 3.67	3.20 3.19
Wechsler (-)	None					

Controls Only

Condition	Region	Atlas	P_{FWE}	K_E	*T* value	*Z* value
Cortisol (+)	None					
Cortisol (-)	None					
BDI (+)	R Middle orbito frontal gyrus (26 53 -12)	aal	0.030	45	3.84	3.30
	Left Inferior parietal lobule (-50 -43 54)	aal	0.024	227	4.57	3.76
	L Inferior temporal gyrus (-39 -37 -17)	aal	0.042	152	4.43	3.08
BDI (-)	None					
BAI (+)	L Middle frontal gyrus (-33 21 34)	aal	0.049	194	4.52	3.73
	R Hippocampus (21 -30 -8)	aal	0.028	73	4.49	3.72
BAI (-)	None					
Wechsler (+)	L Superior frontal gyrus (-27 60 9) L Superior frontal gyrus (-15 60 19) L Superior frontal gyrus (-15 59 33) L Superior frontal gyrus (-24 38 36)	aal	0.002	1313	6.09 5.27 4.10 4.74	4.57 4.16 3.48 3.86
	L Putamen (-23 -91 16)	aal	0.009	309	4.54	3.75
	L Cuneus (-12 -88 15) L Cuneus (-8 -103 10) L Cuneus (-20 -105 6)	TD labels	0.006	697	4.67 3.64 3.66	3.82 3.64 3.19
	R Superior occipital gyrus (23 -91 16)	aal	0.022	179	4.51	3.73
Wechsler (-)	None					

[a]Tables show ROI investigation at threshold 0.001 uncorrected, P_{FWE} = 0.05 cluster-wise correction and k>10. N= 23 patients, 23 controls, except cortisol with 12 patients and 15 controls. (R = right, L = left; (+) = positive, (-) = negative)

Table III. **Significant[a] t-test results comparing patients and controls for white matter**

Patients-Controls

Condition	Region and coordinate	Atlas	P_{FWE}	K_E	*t* value	*z* value
No covariate	None					
Cortisol	None					
Days since surgery	L Middle occipital gyrus (-27 -82 21)	aal	0.069	17	3.53	3.29
	R Parahippocampus (21 -13 -27)	aal	0.037	11	3.38	3.16
BDI	R Parahippocampus (23 -12 -32)	aal	0.029	64	3.81	3.52
BAI	R Parahippocampus (23 -12 -33)	aal	0.038	10	3.36	3.15
Wechsler	None					

Controls - Patients

Condition	Region	Atlas	P_{FWE}	K_E	*t* value	*z* value
No covariate	L Precentral gyrus (-57 -15 33)	TD label	0.042	117	3.83	3.54
	L Postcentral gyrus (-57 -15 33)	aal	0.026	374	4.27	3.88
	L Precuneus (-26 -66 40)	TD label	0.048	100	4.05	3.71
	R Inferior Frontal Operculum (39 8 22)	aal	0.031	54	4.02	3.69
	L Insula (-35 11 9)	TD label	0.010	661	3.90	3.60
	L Inferior Frontal Operculum (-41 12 12)	aal	0.010	432	3.87	3.57
	L Putamen (-21 18 4)	aal	0.025	35	3.37	3.16
	R Supramarginal (62 -28 25)	aal	0.034	77	3.68	3.42
	R Middle temporal gyrus (33 -66 28)	TD label	0.087	46	3.57	3.33
	L Corpus Collosum (-3 20 -0)	TD Br.	0.043	20	3.46	3.24
	L Inferior Parietal lobule (-45 -49 49)	aal	0.046	37	3,43	3.21
	L Caudate (-11 5 18)	aal	0.043	20	3.38	3.17
Diurnal cortisol	L Middle temporal gyrus (-54 -67 15)	aal	0.036	282	4.86	4.01
	L Precentral gyrus(-57 -12 33)	TD labels	0.042	228	4.40	3.73
	L Postcentral gyrus (-57 -12 33)	aal	0.028	333	4.40	3.73
	R Superior frontal gyrus (17 66 7)	aal	0.060	137	4.08	3.51
	R Superior frontal gyrus (24 62 1)				3.90	3.40
	R Inferior Parietal, Angular (44 -66 37)	aal	0.042	29	3.67	3.24
	R Middle occipital gyrus (32 -67 33)	aal	0.049	33	3.61	3.20
Days since surgery	L Superior temporal pole (-39 6 -17)	aal	0.034	21	3.57	3.32
	L Insula (-29 17 1)	aal	0.045	27	3.52	3.28
BDI	L Inferior Parietal lobule (-54 -57 40)	aal	0.017	397	4.07	3.72
	L Parietal lobule, Angular (-54 -57 40)	aal	0.020	106	4.02	3.69
	L Precentral gyrus (-57 -15 33)	TD label	0.050	163	3.92	3.60
	L Postcentral gyrus (-57 -15 33)	aal	0.038	217	3.92	3.60
	L Precuneus (-26 -66 39)	TD label	0.058	51	3.70	3.43
	R Supramarginal gyrus (62 -49 25)	aal	0.034	78	3.66	3.40
	L Inferior frontal operculum (-44 14 13)	aal	0.027	49	3.39	3.17
BAI	L Inferior parietal lobule (-45 -49 49)	aal	0.009	729	4.35	3.94
	L Inferior parietal lobule (-53 -60 42)				4.25	3.86
	L Precentral gyrus (-56 -15 33)	TD label	0.053	72	3.92	3.52
	L Postcentral gyrus (-56 -15 33)	aal	0.035	61	3.81	3.61
	L Insula (-35 9 9)	aal	0.022	246	3.56	3.32
	R Inferior frontal operculum (39 8 22)	aal	0.039	10	3.50	3.27
	R Supramarginal gyrus(63 -46 25)	aal	0.046	13	3.45	3.22
Wechsler	L Precentral gyrus (-56 -15 31)	TD label	0.043	218	4.30	3.90
	L Postcentral gyrus (-56 -15 31)	aal	0.026	383	4.30	3.90
	L Insula (-42 12 12)	TD label	0.014	492	3.86	3.55
	L Inferior frontal operculum (-42 12 12)	aal	0.012	346	3.86	3.55
	L Precuneus (-26 -66 40)	TD label	0.053	72	3.82	3.52
	L Middle occipital gyrus (-26 -66 40)	aal	0.060	49	3.82	3.52
	R Inferior frontal operculum (39 8 22)	aal	0.037	19	3.66	3.40

[a]Tables show significant ROI investigation at threshold 0.001 uncorrected, P_{FWE} = 0.05 cluster-wise correction and k>10.. N= 23 patients and 23 controls, except cortisol with 12 patients and 15 controls. (R = right, L = left)

Table IV. **Significant[a] regression results for white matter in all participants, patients and controls**

All participants

Condition	Region	Atlas	P_{FWE}	K_E	*T* value	*Z* value
Cortisol (+)	L Precuneus (-5 -63 52)	TD labels	0.055	84	3.95	3.45
	L Superior occipital gyrus (-18 -91 31)	TD labels	0.052	46	3.93	3.43
Cortisol (-)	None					
BDI (+)	L Inferior temporal gyrus (-57 -24 -26)	aal	0.028	286	3.92	3.61
	L Superior temporal pole (-50 8 -12	aal	0.025	100	3.92	3.61
BDI (-)	R Inferior frontal operculum (39 6 24)	aal	0.020	187	4.58	4.12
	L medial frontal gyrus (-12 45 15)	TD labels	0.010	1085	4.11	4.11
	L medial frontal gyrus (-6 59 21)				3.82	3.82
	Right Superior frontal gyrus (15 66 10)	TD labels	0.042	356	4.65	4.17
	Right Superior frontal gyrus (23 51 3)				3.89	3.59
	Right Superior frontal gyrus (6 62 3)				3.62	3.37
	R Anterior cingulate (9 36 16)	aal	0.012	356	3.83	3.54
	R Anterior cingulate (12 44 3)				3.74	3.47
	L Anterior cingulate (-6 18 30)	aal	0.020	209	4.57	4.11
	L Anterior cingulate (-8 27 25)				3.88	3.58
	L Insula (-38 -22 16)	TD labels	0.038	80	3.58	3,34
	R Caudate (18 23 10)	aal	0.025	48	3.41	3.20
	L Superior parietal lobule (-17 -54 61)	TD labels	0.026	30	3.62	3.32
	L Precuneus (-24 -58 52)	TD labels	0.044	133	3.92	3.61
	R Anterior cerebellar culmen (0 -51 -18)	TD labels	0.052	34	3.43	3.21
	L Posterior cerebellar declive (3.-63 -18)	TD labels	0.034	72	3.47	3.25
	L Posterior cerebellar tonsil (-23 -48 -47)	TD labels	0.037	31	3.40	3.18
BAI (+)	L Angular gyrus (-42 -60 39)	aal	0.027	35	3.46	3.23
BAI (-)	L Inferior orbito-frontal (-48 36 -6)	aal	0.025	117	3.74	3.47
Wechsler (+)	R Precentral gyrus (50 -15 59)	aal	0.033	246	4.32	3.92
	R Postcentral gyrus (54 -25 51)	aal	0.021	508	3.97	3.65
	R Precuneus (21 -57 48)	aal	0.045	130	3.75	3.48
	L Cuneus (-5 -73 4)	TD labels	0.024	281	4.31	3.91
Wechsler (-)	None					

Patients only

Condition	Region	Atlas	P_{FWE}	K_E	*T* value	*Z* value
Days since surgery (+)	L Insula (-32 11 -18)	aal	0.029	156	4.24	3.57
	L Insula (-39 5 -14)				3.97	3.39
	L Putamen (-29 12 -0)	aal	0.028	19	3.68	3.20
Days since surgery (-)	R Inferior frontal triangularis (56 20 4)	aal	0.042	34	3.89	3.34
Cortisol (+)	R Middle frontal gyrus (33 39 -11)	TD labels	0.009	791	5.98	3.81
	R Middle frontal gyrus (27 42 -17)				5.78	3.75
	R Middle frontal gyrus (32 50 -5)				5.45	3.63
	R Middle frontal gyrus (36 35 -5)				5.07	3.49
	R Superior orbito-frontal (11 45 -23)	aal	0.029	90	5.26	3.56
	R Cingulate gyrus (9 -28 33)	TD labels	0.025	250	5.57	3.68
	R Cingulate gyrus (2 -24 31)				5.31	3.58
	L Rolandic Operculum (-47 -4 16)	aal	0.020	141	5.27	3.57
	L Anterior cerebellar culmen (-36 -45 -36)	TD labels	0.033	171	4.96	3.44
	L Anterior cerebellar culmen (-33 -54 -32)				4.94	3.44
Cortisol (-)	None					
BDI (+)	None					
BDI (-)	R Inferior frontal triangularis (39 35 6)	aal	0.031	110	4.23	3.56
	R Brainstem (2 -19 -20)	TD labels	0.033	121	3.78	3.27
	R Brainstem (12 -16 -23)				3.64	3.17
BAI (+)	L Angular gyrus (-44 -58 39)	aal	0.027	46	3.84	3.30
BAI (-)	L Superior medial frontal gyrus (-11 45 43)	aal	0.048	73	4.16	3.51
Wechsler (+)	None					
Wechsler (-)	L Inferior orbito-frontal gyrus (-42 23 -15)	aal	0.041	14	3.64	3.17
	R Corpus callosum (2 -30 12)	TD Br.	0.043	31	3.68	3.19
	L Corpus callosum (-2 -30 12)	TD Br.	0.043	23	3.68	3.20

Table IV (cont.) **Significant[a] regression results for white matter in all participants, patients and controls**

Controls Only

Condition	Region	Atlas	P_{FWE}	K_E	*T* value	*Z* value
Cortisol (+)	None					
Cortisol (-)	R Parahippocampus (35 -27 -26)	TD labels	0.036	140	5.49	3.88
BDI (+)	None					
BDI (-)	L Middle frontal gyrus (-24 -3 52) L Middle frontal gyrus (-36 -4 52)	TD labels	0.020	718	5.00 4.05	4.01 3.45
	L Precentral gyrus (-9 -28 75)	TD labels	0.035	288	4.33	3.62
BAI (+)	L Posterior cerebellar declive (-8 81 -21)	TD labels	0.039	66	4.30	3.60
BAI (-)	None					
Wechsler (+)	R Posterior cingulate gyrus (9 -67 12) R Posterior cingulate gyrus (2 -70 10)	TD labels	0.019	107	3.95 3.58	3.38 3.12
	R Caudate (17 18 10) R Caudate (15 3 21)	aal	0.005	605	4.97 4.18	4.00 3.53
	R Precuneus (21 -57 51) R Precuneus (6 -64 15)	aal	0.025	334	5.28 3.88	4.17 3.33
	L Cuneus (-2 -79 21)	aal	0.036	56	3.74	3.28
Wechsler (-)	None					

[a]Tables show significant ROI investigation at threshold 0.001 uncorrected, P_{FWE} = 0.05 cluster-wise correction and k>10.. N= 23 patients, 23 controls, except cortisol with 12 patients and 15 controls. (R = right, L = left; (+) = positive, (-) = negative)

Figure 1- **Diurnal cortisol rhythms for breast cancer patients and matched controls**

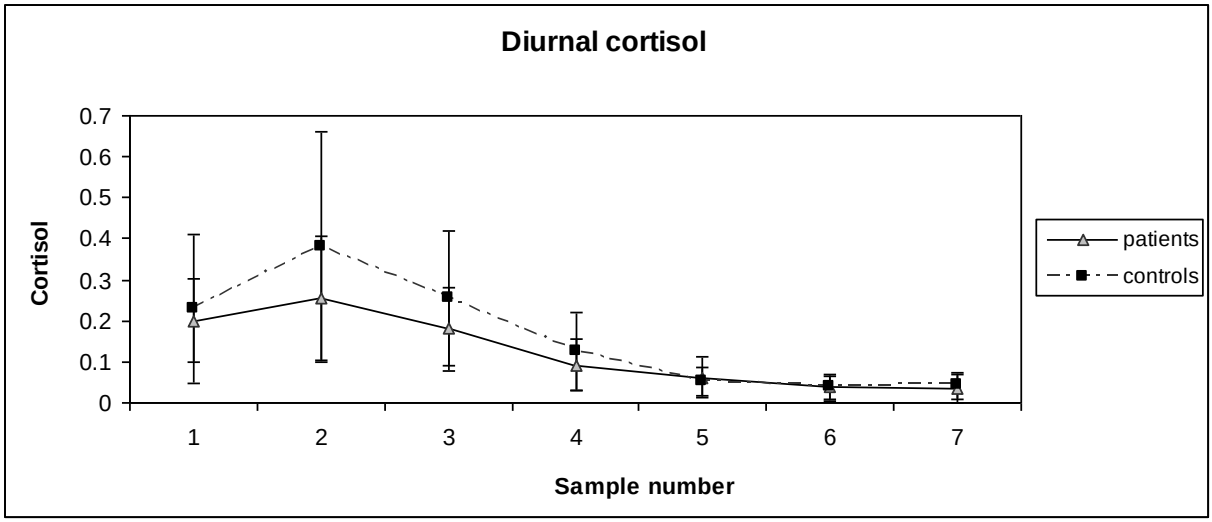
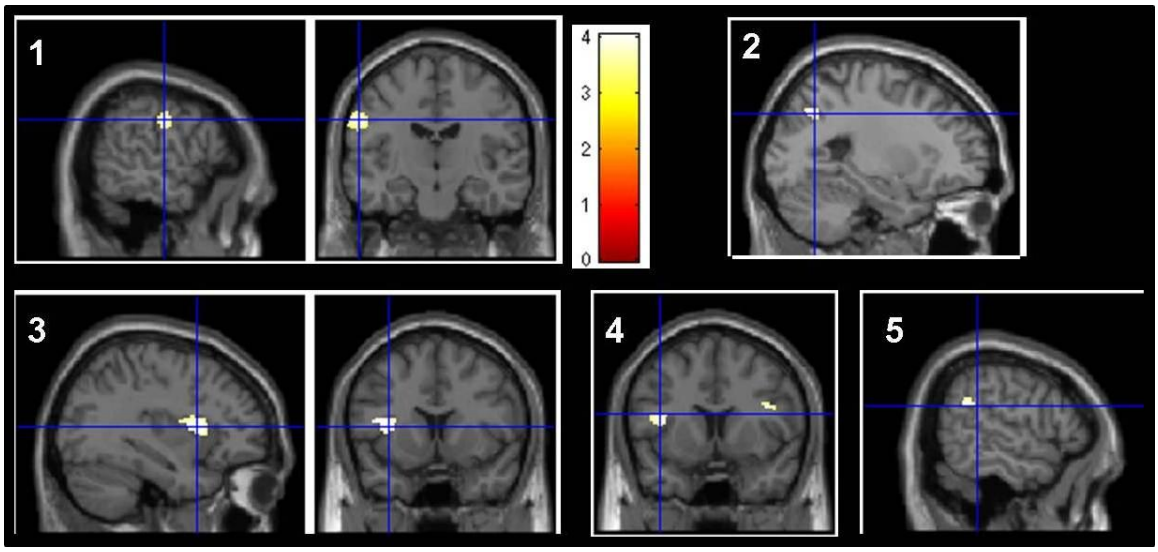


Figure 2- **Significant t-test results for controls – patients for white matter**



1. Left precentral and postcentral gyri
2. Left precuneus
3. Left insula
4. Bilateral inferior frontal operculum
5. Right supramarginal gyrus

Chapter 4

Title: Pre-chemotherapy differences in visuospatial working memory in breast cancer patients compared to controls: An fMRI study.

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Abstract

Introduction

Cognitive deficits are a side-effect of chemotherapy, however pre-treatment research is limited. This study examines neurofunctional differences during working memory between breast cancer (BC) patients and controls, prior to chemotherapy.

Methods

Twenty-three early-stage BC females, scanned after surgery but before chemotherapy, were matched to non-cancer controls. Participants underwent fMRI while performing a Visuospatial N-back task and data was analyzed by multiple group comparisons. fMRI task performance, neuropsychological tests, hospital records and salivary biomarkers were also collected.

Results

There were no significant group differences on neuropsychological tests, estrogen or cortisol. Patients made significantly fewer commission errors but had less overall correct responses and were slower than controls during the task. Significant group differences were observed for the fMRI data, yet the results depended on the type of analysis. BC patients presented with increased activations during working memory compared to controls in areas such as the inferior frontal gyrus, insula, thalamus, and midbrain. Individual group regressions revealed a reverse relationship between brain activity and commission errors.

Conclusions

This is the first fMRI investigation to reveal neurophysiological differences during visuospatial working memory between BC patients pre-chemotherapy and controls.

Significance

This highlights the need to better understand the pre-chemotherapy BC patient and the effects of associated confounding variables.

Keywords:

Chemotherapy, breast cancer, cognitive impairment, functional magnetic resonance imaging, visuospatial working memory, pre-treatment effects.

Highlights

- Prior to chemotherapy, BC patients compared to controls show increased activity related to visuospatial working memory in the left inferior frontal operculum and right midbrain, which is modified by the addition of task reaction times and suspected confounding variables, such as depression.
- Contrasts of active task and rest conditions reveal both increased and decreased widespread neural activations in patients compared to controls, suggesting that there are group differences during active task engagement and/or at rest.
- Independent regression analyses in both patients and controls reveal an opposite relationship between neural activation and errors of commission, with patients showing more activity with more erroneous answers. Yet patients reveal overall lower task performance and slower reaction times indicating they are potentially being more cautious than controls.

Introduction

Cognitive deficits, particularly in executive functioning domains such as working memory, are often reported in chemotherapy-treated cancer populations. Such dysfunctions are increasingly described in adjuvant-chemotherapy breast cancer (BC) populations and are as such garnering more attention from the scientific community. Long-term survival is more frequently achieved with recent medical advances and is the primary focus in cancer populations. Meanwhile, secondary negative effects from treatment can potentially persist for years and considerably affect quality of life. To describe these self-reported cognitive deficits manifesting during and after chemotherapy, patients have coined the terms “chemofog” or “chemobrain”. The reported incidence of chemotherapy-related impairments is highly variable with a range of 17% to 75% across studies 0. Even with this inconsistency, these self-reports have been validated by neurospychological studies, which have largely confirmed that chemotherapy-treated patients perform more poorly on neurocognitive tests compared to non-exposed controls [0-0]. Additionally, neuroimaging studies have shown both structural and functional differences between patients and controls, even if the performance differences between the groups are very subtle [0-[35]]. In particular, these self-reported deficits, corroborated by neuropsychological and neuroimaging studies, are manifested mostly in domains such as processing speed and executive functioning, including working memory.

Working memory refers to the processes required to store and manipulate information for a short amount of time, subsequently followed by its retrieval [[36], [37]]. This is an important component of executive functioning, requiring both the integrity of the prefrontal cortex, as well as its connections to the rest of the brain [[38], [39]]. The hippocampus is one of those associated areas required in several types of memory, including spatial memory [40]; visuospatial memory is particularly important due to its utility in day to day living. In addition to the frontal cortex and hippocampus, working memory is also subserved by the parahippocampal gyrus, the posterior parietal cortex, precuneus and fusiform gyrus [[41]-[45]].

Memory and attention have been target domains in neuroimaging studies of chemofog, with differences revealed between chemotherapy-treated patients (chemo+) and controls in frontal regions as well as associated areas. First, a delayed-recall word

memory task during a [O-15] PET scan 0 revealed that chemo+ BC patients (minimum 5 years after treatment completion, self-reports of cognitive deficits) activated a larger portion of the inferior frontal cortex compared to other treatment BC patients (chemo-) and healthy participants. Interestingly, a [F-18] fluorodeoxyglucose-PET assessed lower resting brain metabolism in the left frontal gyrus and the contralateral cerebellum in chemo+ patients compared to controls. Additionally, lower resting metabolism in the frontal cortex correlated with the more impaired memory task performance. The authors suggest that the frontal cortex in chemo+ patients has to work harder to successfully perform the task. Second, an fMRI study 9 applied a working memory task in chemo+ BC patients, local therapy only BC patients and healthy participants. Working memory was assessed before and 1 month after treatment using an auditory N-back task with variable processing load requirements (0, 1, 2, and 3-back conditions). No group differences were observed at baseline, with the expected pattern of frontal, parietal and cerebellar activations apparent during the most challenging load. Post-treatment fMRI assessments in chemo+ patients compared to both control groups uncovered increased activation in posterior frontal and parietal regions, as well as less bilateral activity in more anterior frontal regions. Third, the largest and most recent fMRI study [34] applied two executive functioning tasks in chemo+ (10 years post-treatment) and chemo- BC survivors. Chemo+ patients performed more poorly and had quicker reaction times while completing a Tower of London task compared to controls. Chemo+ patients additionally showed dorsal lateral prefrontal cortex (DLPFC) hyporesponsiveness and decreased activation in bilateral posterior parietal cortex. A paired-associates task was the second assessment and measured episodic memory. Chemo+ patients revealed hyporesponsiveness of the parahippocampal gyrus and the bilateral lateral posterior parietal cortex, left precuneus, right dorsal striatum, right inferior parietal cortex and left middle temporal gyrus compared to controls. This study suggests long-term effects of chemotherapy on executive functioning, linking DLPFC hypoactivation with impaired planning behaviour often seen in chemo+ patients as well as increased impulsivity due to impaired attentional abilities.

Therefore, there are functional neural differences related to executive functioning between a chemotherapy-treated patient population and varied control groups, and one

focused domain that seems to be affected is working memory. However, while both the term “chemofog” as well as the current research focus suggest a direct causal link between chemotherapy and cognitive deficits, other factors should be considered. An array of factors may contribute to variability between studies: nature of the control group, assessment tasks used, cross-sectional versus prospective studies, effects of anesthesia, and stress of cancer diagnosis. One study 0 has revealed pre-treatment group differences when conducting an fMRI study of verbal working memory in healthy controls and BC patients prior to chemotherapy. The authors revealed that patients had slower reaction times in the high-demand condition of the task as well as less accuracy in their responses. With increased cognitive demand, patients also showed larger activation in the right inferior frontal gyrus as well as additional components of attention/working memory circuitry in both hemispheres compared to controls. This study 0 highlights the importance of assessments of cancer patients before treatment as it suggests pre-treatment cognitive deficits. Without appropriate baseline assessments and scans, the effects of the disease itself or other baseline confounding variables could be mistaken for an effect of treatment. Similarly, these deficits could go undetected and lead to problems in day to day living for these women given the importance of executive functioning in such things as decision making and memory.

More attention to baseline differences between cancer patients and controls is warranted in order to tease out the contribution of other factors, either manifesting before chemotherapy commencement or exacerbating its effect. An important factor to consider is the presence of cancer itself, controlled by matching chemotherapy-treated patients (chemo+) to another cancer group receiving a different treatment (chemo-) [7, 9, [34]]. This practice is even more useful if the chemo- cancer group is applied as a second control group, along with healthy controls [7, 9]. However, the chemo+ and chemo- pairing presents differences in treatment. For example, the chemo- group may receive radiation treatment or anti-estrogen regimens, both which may contribute to fatigue [47] and be implicated in cognitive dysfunction in BC patients 0. Regardless, the presence of cancer is an important factor to consider as ongoing studies have revealed elevated cytokine levels in cancer patients [48] compared to healthy controls. These small proteins both interact with and modulate the activity of other cells in the body leading to

inflammation, tissue destruction and/or disease. Of particular note is the problem of memory consolidation reported with high cytokine activity in the hippocampus [49]. The effects of cytokines can persevere for 6-60 months after diagnosis, with values being highest post-surgery [48].

In addition to elevated cytokine levels post-surgery, cognitive decline linked to general anaesthesia has been suggested. The latter is particularly potent in elderly patients (60+) who are more prone to develop long-term cognitive deficits [[50]-[52]]; exactly the age range of many cancer patients, especially in a BC population. Hence, cognitive deficits manifesting as side-effects of surgery that are not identified at baseline could be misattributed as chemofog deficits, especially in cross-sectional studies. One imaging study 7 applied a covariate of "days between surgery date and baseline MRI scan" in a comparison between chemo+ and chemo- groups. The covariate attenuated the statistical model but did not change the results, which led the authors to suggest that this speculated variable does not influence grey matter volume. However, this study compared two groups who had undergone surgery, both possibly exhibiting the same profile of elevated cytokine levels and deficits related to general anaesthesia. More research is needed to understand post- surgery effects in cancer patients compared to healthy controls, and in a larger sample.

Another important factor to consider is the stress experienced by patients as a result of a cancer diagnosis and the follow up medical attention that then occurs. Stress, routinely measured in chemofog studies through self-reports, highly correlates with complaints of cognitive difficulties [0, 0, 0, 0, 0, 0, [52], [54]]. With elevated stress, there is increased activity of the hypothalamic-pituitary-adrenal (HPA) axis which leads to increased glucocorticoid (GC) levels in the body (cortisol in humans). Increased GC levels (natural or a consequence of therapeutic GC regimens that are part of the cancer-related immunosuppressive therapy) have been shown to affect brain anatomy [[55]-[57]] as well as memory functions, such as working memory [58]. In particular, regions in the brain such as the hippocampus are particularly affected by GC-mediated excitotoxicity as a consequence of its elevated number of GC receptors. In fact, smaller hippocampal volumes seen in high stress populations suggest potential negative effects of higher circulating GCs [[59]-[61]]. One chemofog MRI study revealed transient smaller

volumes in key cognitive regions such as the hippocampus in chemo+ BC survivors compared to those treated with surgery alone. These structural differences were mirrored by lower scores in attention, visual memory, and concentration 1 year after chemotherapy 4. Additionally, GCs may have an indirect role in the development of cognitive deficits post-chemotherapy. Higher levels of circulating GCs during high stress, such as the occurrence of a cancer diagnosis and/or treatment, may contribute to increased capillary permeability of the blood brain barrier [62] which could lead to increased accessibility of chemotherapy agents to the brain. Therefore, increased GC levels could potentially exacerbate the adverse effects of chemotherapy on the brain.

Finally, another biological factor of importance in a typically older BC population is estrogen levels as chemotherapy-treatment is known to trigger early-onset menopause [[63], [64]] and many women are already post-menopausal at the time of diagnosis. Estrogen has long been considered to have a neuroprotective role in the brain, thereby helping maintain cognitive capacities. While the exact mechanism by which it exerts such protection remains unclear, there is likely an interaction between various neurotransmitter systems, neurotrophic factors as well as nuclear estrogen receptors. These are particularly present in areas such as the hippocampus and amygdala, both implicated in learning and memory [[65]-[67]]. After menopause, women report declining cognitive capacities and show decreased bilateral hippocampal volumes. This effect has been shown to be minimized by estrogen therapy [68].The current study examined functional neural processing in newly diagnosed female BC patients (prior to chemotherapy commencement) compared to healthy controls completing a visuospatial working memory task. The visuospatial n-back task is a commonly-used fMRI compatible cognitive assessment utilized to investigate visuospatial working memory and has been successfully applied in both healthy and clinical populations [[69]-[74]]. Brain regions that are particularly important for successful visuospatial working memory in healthy populations include the frontal cortex, hippocampus, parahippocampal gyrus, posterior parietal cortex, precuneus, fusiform gyrus, and cerebellum [[41]-[45], [75]]. A variety of speculated pre-chemotherapy confound variables were considered as covariates in the fMRI analysis, including: days since surgery, diurnal cortisol levels, estrogen measurements, depression and anxiety inventories, and task performance scores. It was

hypothesized that addition of the above covariates may modify the analyses with no covariates (as seen in previous studies 7), yet these may also uncover new baseline differences between patients and controls and/or expose heterogeneity within the patient sample itself. Such results would emphasize the multifactorial nature of the BC population and the need to include baseline comparisons to fully understand the repercussions of chemotherapy on cognitive processing.

Methods

Twenty-three early stage breast cancer (BC) patients were recruited through the Ottawa Hospital Regional Cancer Centre (average age: 51 ± 8.5 years, range: 35-64). Patients had undergone mastectomy or lumpectomy and were scheduled to commence a chemotherapy regimen. Inclusion criteria were: female, no previous cancer or cancer treatment, no unstable psychiatric/neurological illness, no history of substance abuse, scores of <20 on the Beck depression inventory (BDI-II; [76]) and <15 on the Beck anxiety inventory (BAI; [77]), fluency in English, and minimum grade 8 education. fMRI exclusion criteria included left-handedness, sight problems, claustrophobia, pacemaker, and metal implants. Healthy controls (average age: 49 ± 9 years, range: 30-62) were either nominated by a patient or recruited through posters/website ads and were each sex-, age- and education-matched to individual BC patients. Non-cancer controls also met the above inclusion and exclusion criteria and followed the same procedure as their index patient.

Neuropsychological Assessments:

Each participant completed psychometric tests and MRI/fMRI scanning sessions. The 2 ½ hour psychometric test battery included: social /medical history, self-report questionnaires of physical, emotional, and cognitive functioning, traditional pencil-and-paper neuropsychological tests, and a 30 minute computerized cognitive test (CNSVS; https://www.cnsvs.com/). This computerized test, validated [78] in a broad age range and across clinical and non-clinical populations (incl. cancer patients), has also been proven sensitive in monitoring patient's cognitive status over time. In particular this battery

measures: attention, reaction time, working memory, executive function, visual episodic memory, and verbal episodic memory. Raw neuropsychological data were converted to standardized scores, based on the means and standard deviations of the healthy matched controls group. Summary scores were computed for several cognitive domains, as well as an overall cognitive summary calculated by averaging all the cognitive test scores.. Cognitive domains were determined based on both rational and empirical grounds. Nineteen tests comprised in the neuropsychological battery from which the 6 following cognitive domains were extracted: 1. Verbal Memory (Hopkins Verbal Learning Test-Revised (HVLT-R; [79] and CNSVS Verbal Memory Index), 2. Processing Speed (WAIS-III [80]) Digit-symbol coding, WAIS-III Symbol Search, controlled oral word association; [81]), CNSVS Processing speed index, CNSVS Executive Functioning index, CNSVS Reaction time index and CNSVS Cognitive Flexibility index), 3. Attention (WAIS-III Digit Span, WAIS-III Letter-number sequencing, Paced Auditory Serial Attention Test (PASAT; [82]), CNSVS Sustained attention index and Trailmaking Test, Part B [83]), 4. Visual Memory (CNSVS Visual Memory index, Brief Visuospatial Memory Test-Revised (BVMT-R; [84]) and CNSVS Working Memory index), 5. Reasoning (CNSVS Reasoning index) and 6. Verbal Short- term Memory (Auditory Consonant Trigrams; [85]). At the baseline scanning session, each participant also completed the Rosenberg Self-esteem questionnaire (RSE; [191]) and the Questionnaire for Competence and Control (FKK; [87]).

Demographic, clinical neuropsychological and self-esteem data were analyzed by 2-tailed independent t-tests using Statistical Package for the Social Sciences version 18.0 program (SPSS v. 18.0, Chicago, IL). A correlational analysis was also conducted to reveal any relationships between factors.

Assessment of biological markers:

Baseline blood work (hemoglobin, white blood cell and neutrophil counts) were obtained from patient's hospital records. Diurnal free cortisol levels were assessed via salivary sampling [193]. Each at-home sampling kit used for this study contained 14 cotton salivettes for cortisol sampling and detailed instructions. Participants were instructed to follow a rigid sampling schedule for 2 consecutive and typical weekdays.

Cortisol was sampled at 7 time points for each of the 2 days at: Awakening, 30 min after awakening, 1 hour after awakening (after this sample, participants may consume food and drink), 10am, 2pm, 6pm and 9pm. Such a sampling schedule provides a circadian cortisol profile for each participant (patients and controls), an important factor to consider since cortisol varies according to circadian rhythms and such cycles are seen to be affected in high stress and clinical populations [[194]-[198]]. Participants were instructed to keep the completed samples in a freezer until the next appointment. The saliva samples were analyzed for cortisol using ELISA techniques (as described by Salimetrics at salimetrics.com). Intra- and inter-assay variability (CVs) for datapoints (excluding outliers) were less than 10% and 15%, respectively. The area under the curve from ground (AUCg) calculation was computed in accordance to the formulas described by Pruessner [199] in order to analyze salivary cortisol differences. AUCg was computed and 2-tailed independent t-tests were applied using SPSS v. 18.0.

Estrogen samples were also collected as part of the home sampling kits by collecting pure spit. This was scheduled to be completed at the 10 am mark for each of the 2 consecutive days. The saliva samples were analyzed for estrodiol using ELISA techniques (described by Salimetrics at salimetrics.com). Intra-assay coefficients of variation on one estrogen measurement / participant were less than 0.43 (CV (%) = 4.03, Max: 19 and Min 0.148). Group differences in estrogen levels were assessed by a 2-tailed independent t-test using SPSS v. 18.0.

Scanning sessions:

Brain images were acquired using a 1.5 Tesla Siemens Magnetom Symphony MR scanner. Subjects were instructed to lie on their back with their head secured in a standard head holder and to stay as still as possible when in the scanner. A gradient echo localizer was acquired and used to prescribe a subsequent 3D FLASH (Fast Low Angle Shot, a spoiled gradient sequence) (TR/TE 22/9.2ms, flip angle 30º, field of view (FOV) 256x256 mm scan). Whole brain echo planar fMRI based on the blood oxygen level dependent (BOLD) effect was performed using a gradient echo pulse sequence (TR/TE 3000/40ms, flip angle 90º, FOV 250x187.5,mm 64x64 matrix, slice thickness 5mm, 27 axial slices, bandwidth 2430 Hz per pixel).

fMRI Visuospatial N-back task:

The task was projected through an LCD projector from a computer in the control room, and presented on a rear projection screen placed near the end of the patient table. A mirrored head coil allowed the participant to view the task while lying in the scanner. In order to increase visibility, all lighting in the scanning room was off. Participant responses, in the form of button presses on a response pad, were recorded via a MRI compatible fiber optic device (Lumina LP-400, Cedrus).

The Visuospatial N-back was a block-design task used to measure visuospatial working memory processes while in the scanner. Stimuli were presented as white circular (O) targets in 9 different positions on a solid black background. Participants were instructed to use the response pad with their right hand, and to press the correct key as quickly and accurately as possible, using the right index finger. They were encouraged not to dwell on previous mistakes. The scanning session began with an initial rest epoch of 9 seconds in order to allow longitudinal magnetic relaxation (T1 effects) to stabilize. Images collected during this initial rest epoch were excluded from the image analyses.

The Visuospatial N-back procedure involved two conditions of target presentations, with the circular stimuli being presented one at a time on the screen for 240 ms (interstimulus interval of 1760 ms). The first condition, 'Press for center O', required the participant to press a button when an O appeared in the center of the screen and to refrain from pressing when it appeared at another location on the screen. The second condition, 'Press for 2-back', required the participant to press when the target appeared at the same location on the screen as the stimuli presented 2 trials prior and to refrain from pressing for all other presentations. Both of these conditions were presented in 12 epochs of 33 seconds. Each epoch began with a 3 second instruction screen, followed by the presentation of 15 stimuli. There were two 24 second rest epochs, one in the middle of the task and another at the very end. During instruction epochs, the participant was to either 'Press for center O' and 'Press for 2-back'. During rest epochs, the word 'REST' was presented on the screen, instructing the participant that no motor response was required. Once in the scanner, six blocks of each condition, 'Press for center O' and 'Press for 2-back' epochs, were presented in a counterbalanced order, starting with the 'Press for

center O' condition. This task lasted 453 seconds. After the completed task, all participants were asked to rate the difficulty of the task on a 5-point Likert Scale to investigate metacognitive capacity.

fMRI Performance Parameters and Analyses

Errors of commission included any incorrect response following a stimulus (ex: pressing a target not presented in the center in the 'Press for center O' condition or pressing for a target that was not presented 2 prior while performing the 'Press for 2-back' condition) within 900 ms of stimulus presentation. Omission errors were defined as a failure to respond to a target stimulus within the same time frame. All accurate responses occurring within 900 ms of stimulus presentation were used to calculate the percent correct scores and the mean reaction times for both conditions.

Reaction times for each response in the scanner, metacognition scores, and both errors of commission and omission (or percent correct scores) were analyzed. Group differences were computed by 2-tailed independent sample t-tests using SPSS v. 18.0. A correlational analysis was also applied to reveal if there was a relationship between the cognitive impression of task difficulty and actual task performance.

Image Processing

Statistical Parametric Mapping 8 (SPM8) was used to post-process the fMRI data and to perform the statistical analyses specific to this dataset. The functional images were reconstructed and whole brain images were realigned to correct for motion by employing the procedure of Friston and colleagues [200]. Then, images were spatially normalized to match the echo planar imaging (EPI) template provided in SPM8. Following spatial normalization, images were smoothed with a 10mm full-width at half-maximum Gaussian filter.

First level analyses were performed for each participant using these images representing the two task conditions and the rests. Motion correction was applied as a regressor for all first level analyses. Visuospatial working memory processes were isolated by subtracting the averaged 'Press for center O' images from the averaged 'Press for 2-back' images (condition labelled as "2back-O"), creating one image per participant.

Two additional contrasts were also computed at the first level: "press for center O – rest" (or "O-rest") and "press for "2back – rest" (or "2back-rest"). These processed within-subject images were then entered into the second level analyses for group comparisons between BC patients and controls. Second level analyses included 3 different assessments. First, a 2-way independent group t-test of the "2back-O" contrast was executed without covariates, and then separate t-tests were performed with suspected confound variables applied as covariates in order to extract their possible individual effects. Second, flexible factorial assessments of both "O-rest" and "2back-rest" contrasts were conducted in order to assess if the rest condition itself could drive group differences. Third, regression analyses along days since surgery in the patient group and fMRI task errors of commission, percent correct, and reaction times in both controls and patients were performed for the "2back-O" contrast to assess if there was group heterogeneity in visuospatial working memory caused by these factors.

Whole brain and ROI investigations for both t-test and regression analyses were conducted at a set threshold of $p_{\text{uncorr.}} = 0.001$, with a cluster-wise correction at $p_{\text{FWE}} = 0.05$ and a set cluster size larger than 10 voxels. Whole brain fMRI, more stringent than ROI analyses, measures activation differences over the entire brain, thus any effect size apparent in small areas will be diluted. ROIs investigated in this study were extracted from several sources including, brain areas reported as affected in previous post-chemotherapy structural and functional studies [4-0, 9, 0], regions related to the covariates used and areas with a role in working memory [72]. Bilateral ROIs considered included: frontal lobe, precentral gyrus, cingulate gyrus, parahippocampus, hippocampus, insula, thalamus, basal ganglia, temporal lobe, parietal lobe, precuneus, occipital lobe, brainstem and cerebellum. Significant results presented are both un-adjusted and adjusted for multiple comparisons. A Bonferroni adjustment with 28 comparisons lowers the alpha to 3.57^{-5} with a significant z-value now of 3.97. Meanwhile, whole brain investigations for flexible factorial analyses were conducted at a set threshold of p= 0.05 corrected, with a cluster-wise correction at $p_{\text{FWE}} = 0.05$.

Results

Demographic and Clinical Data

Correlations were computed to investigate any relationships between the demographic and neuropsychological factors. Age negatively correlated with attention ($r(46) = -0.311$, $p= 0.035$), reasoning ($r(46) = -0.418$, $p= 0.004$), and processing speed ($r(46) = -0.531$, $p< 0.001$), with younger participants scoring higher on tests comprising these cognitive domains. The Wechsler test of Adult Reading positively correlated with 5 out of the 6 computed cognitive domains: attention ($r(46) = 0.486$, $p= 0.001$), visual memory ($r(46) = 0.332$, $p= 0.026$), reasoning ($r(46) = 0.295$, $p= 0.049$), verbal short-term memory ($r(46) = 0.459$, $p= 0.002$), and processing speed ($r(46) = 0.447$, $p= 0.002$). Therefore, higher Wechsler scores predict higher scores in these cognitive domains. Additionally, verbal memory positively correlated with reasoning ($r(46) = 0.353$, $p= 0.016$), attention correlated with visual memory ($r(46) = 0.496$, $p= 0.000$), reasoning ($r(46) = 0.596$, $p= 0.000$) as well as processing speed ($r(46) = 0.551$, $p= 0.000$) and visual memory correlated with processing speed ($r(46) = 0.314$, $p= 0.033$). The BDI revealed a larger mean score in patients (8.3) compared to controls (3.8) ($t(44) = 2.299$, $p= 0.026$). Similarly, the BAI showed larger scores in patients (7.8) compared to controls (4.1) ($t(44)= 2.090$, $p=0.042$). As expected, BDI scores positively correlated with BAI scores ($r(46) = 0.342$, $p= 0.020$). A negative correlation was revealed between BDI scores and the processing speed domain ($r(46) = -0.368$, $p= 0.012$) while a similar negative correlation was shown between BAI scores and verbal short-term memory ($r(46) = -0.362$, $p= 0.013$).

Table A summarizes demographic and clinical characteristics for all participants. Estimated IQ was assessed by the Wechsler Adult Reading Task, and no significant group differences between patients and controls were revealed ($t(44) = -1.312$, $p = 0.197$). No significant differences existed between groups concerning menopausal status, with half the sample being post-menopausal. Finally, there were no significant group differences in any of the 6 cognitive domains or on self-esteem measures.

Biological measures

Patient blood work revealed average values in the normal range: Hemoblobin (HGB): 134 grams/L, White blood cells (WBC): 7 x 10^9/L, Neutrophils: 4.21 x 10^9/L. Independent t-tests between patients and controls did not reveal significant differences on salivary estrogen levels ($t(25) = 0.835$, $p>0.412$) with average values (Std dev) being 6.07 (3.89) and 4.98 (2.89), respectively. Each subject's diurnal cortisol index ranged from 0 to 0.973 ug/dL, mean (Std dev) = 0.144 (0.159). AUCg values of diurnal salivary cortisol ($t(25) = -1.308$, $p > 0.203$) were not significantly different between groups, with average AUCg (Std dev) at 12.7 (5.41) and 16.1 (8.68), respectively for patients and controls. See Figure 1 for graph of diurnal cortisol rhythms for BC patients and matched controls.

fMRI task performance data

Group differences were revealed concerning reaction times for the "Press for 2back" condition ($t(44) = 2.520$, $p < 0.015$) with patients showing slower response times than controls (mean (Std dev): 547.77ms (78.98) and 486.25ms (86.40)). Also, errors of commission scores significantly differentiated patients and controls ($t(44) = -2.320$, $p < 0.025$) with patients making less erroneous presses than controls (mean (Std dev; max, min): 0.39 (0.66; 2, 0) and 1.18(1.49; 5, 0)). Meanwhile, patients committed more errors of omission than controls and performed with 73% and 82% accuracy, respectively. However, this was not a significant difference ($t(44) = -1.779$, $p > 0.082$). No other group differences were revealed. Overall, metacognition scores across groups correlated with errors of commission ($r(46) = 0.467$, $p= 0.001$), percentage of correct responses ($r(46) = -0.362$, $p= 0.013$), BDI ($r(46) = 0.632$, $p= 0.000$) and BAI ($r(46) = 1$, $p= 0.000$).

fMRI task image analysis

2-way independent group t-test (2back-O)

Independent t-test results of the 2back-O contrast (Whole brain *P* values, MNI coordinates, ROI *P* values, region, cluster extent, T and Z values) are presented in Table B, at threshold $P_{uncorr} < 0.001$ with $P_{FWE} = 0.05$ cluster-wise correction. The data was first analysed without covariates. To control for any effects of "contributing" factors, the following were individually added as covariates in the analyses: cortisol, metacognition,

"Press for 2back" condition reaction time, days since surgery, BDI scores, BAI scores and the number of errors of commission (See Figure 2).

Fixed-effects in patients and controls concerning visuospatial working memory are presented in the Table A suppl. (supplementary material). When comparing groups, patients showed increased activity compared to controls in the left inferior frontal operculum and right midbrain. The addition of days since surgery as a covariate eliminated any group differences. Group differences when considering reaction time as a covariate showed increased activation in patients compared to controls in the right super occipital gyrus and bilateral thalamus. Patients revealed increased activity compared to controls in the left insula as well as the right lateral globus pallidus with the addition of BAI scores and in the red nucleus with the addition of BDI scores. When cortisol was added as a covariate, patients showed increased activity in the left thalamus. Finally when metacognitive scores of performance during the task were considered, patients revealed larger activity in the right thalamus compared to controls. Overall, patients consistently revealed larger activity compared to controls, and these regions changed according to the covariate in question.

Flexible Factorial (O-rest and 2back-rest)

All flexible factorial results for the O-rest and the 2back-rest contrasts (Whole brain P values, MNI coordinates, ROI *P* values, region, T and Z values) are presented in Table C, at threshold corrected $P_{\text{FWE-corrected}} < 0.05$ with $P_{\text{FWE}} = 0.05$ set for multiple comparisons. See Figure 3.

There was an overlap in the regions where patients demonstrated more neural activity compared to controls in both the 0-rest and the 2back-rest conditions. However, the 2back–rest condition additionally showed increased activity in the left hippocampus, right supramarginal gyrus, right angular gyrus, right thalamus, right lingual gyrus and right precuneus in patients compared to controls.

There was also overlap in brain regions where patients showed significantly less activation compared to controls for both the 0-rest and the 2back-rest conditions. However, there were additional, non-overlapping regions for each condition as follows. For the 0-rest condition, there was increased activity in the right precentral, left lentiform

nucleus, right putamen, left middle occipital gyrus, right cuneus and left cerebellar tonsil. For the 2back-rest condition, there were additional differences in the right insula, left middle temporal gyrus, left superior temporal gyrus and left superior parietal lobule.

Regression (2back-O)

Regression results of the 2back-O contrast along days since surgery as well as fMRI task errors of commission, reaction times, and percent correct (Whole brain *P* values, MNI coordinates, ROI P values, region, cluster extent, T and Z values) are presented in Table D, at threshold $P_{uncorr} < 0.001$ with $P_{FWE} = 0.05$ cluster-wise correction. Within group variability in visuospatial working memory was revealed in both groups via regressions along errors of commission with widespread activations. However, while patients mostly revealed positive correlations between brain activity and these errors (more activity was associated with more errors), controls revealed negative correlations (more activity was associated with less errors). Regressions along percentage of correct responses was only significant in the control group, with visuospatial working memory activity increasing in the left inferior frontal gyrus, superior and medial orbitofrontal gyrus as well as parahippocampus with increased performance.

4. Discussion

Post-chemotherapy cognitive impairments reported by cancer patients are widely acknowledged and these can seriously affect quality of life. While post-chemotherapy investigations are becoming more common, only a few studies have investigated and reported on existing pre-treatment cognitive differences [0, [48]]. The purpose of the current study was to further pre-chemotherapy baseline investigations through the examination of neural processing during visuospatial working memory in BC patients and well-matched healthy controls. This was the first study to investigate visuospatial working memory abilities in BC patients using an N-back task in an fMRI setting. Also, this study is novel in its approach to control for multiple variables that may contribute to group differences in cognitive processing prior to chemotherapy.

Working memory deficits are apparent after chemotherapy [0, 9], however, the results from this visuospatial working memory study suggest that these deficits are

manifesting at baseline. Carry-over of these effects to post-treatment assessments could lead to them being mistakenly attributed as a side-effect of chemotherapy. It is also apparent from these study results that the BC population is a multifaceted group requiring consideration of many potentially confounding variables. It is possible that the combination of these variables with chemotherapy-treatment could exacerbate already existing deficits. Therefore, before post-chemotherapy deficits are assessed in BC patients, it is essential to better understand these pre-treatment patients.

This study demonstrates that BC patients, prior to chemotherapy, have different patterns of neural activity than controls when completing a visuospatial working memory task. The particular results vary depending on the statistical protocol implemented and the covariates included, highlighting the need for rigorous analyses procedures. The rigorous analyses conducted in this study collectively enabled a systematic investigation of the many variables playing a role at baseline. Such varied statistical investigations have not been applied in previous studies of BC patients.

An ROI analysis t-test, focused on regions reported by post-chemotherapy studies, uncovered that BC patients show more neural activity related to visuospatial working memory in the left inferior operculum and right midbrain compared to controls. Activation of the inferior frontal gyrus has been reported in the visuospatial n-back task for the 2back-0back analysis [[97]-[99]] as well as the insula (at the frontal operculum) [99]. The left inferior frontal gyrus shows greater activation in working memory tasks with long delays compared to short delays, thereby indicating that this region activates more with increased challenges to working memory processing [100]. Additionally the pars opercularis of the inferior frontal gyrus is active when people suppress motor responses [101], and stimulation causes changes in motor cortical activity [102]. Overall, increased activation in the frontal operculum is interesting as patients reveal slower reaction times compared to controls. This may indicate that patients find the task more challenging than controls (as suggested by the percent correct scores), leading to recruitment of this region to suppress motor responses in order to increase task accuracy. A well-studied neuro-modulatory system related to the prefrontal cortex, in order to adjust to specific tasks, is the dopaminergic input from the midbrain (particularly in the ventral tegmental area and substantia nigra) [103]. Previous research has indicated that

dopaminergic activity increases during working memory tasks [104], [105], which could help explain why patients also show increased midbrain activity compared to controls when completing this task, along with their increased frontal activity.

Of particular interest is the addition of "reaction time" as a covariate, which removed right midbrain differences between groups, and modified the results to reveal increased activity in bilateral thalamus and right superior occipital gyrus in patients compared to controls. Thalamic activations have been linked to performance in attention-demanding reaction time tasks [106]. Considering the close proximity/overlap of the left inferior frontal operculum and insula coordinates, activity in this region was not affected by the addition of reaction time as a covariate. Also, depression and anxiety scores were significantly different between groups (although both in the mild range), yet only BDI scores modified group differences. The addition of BDI scores as a covariate adjusted the findings to uncover that there was no longer left inferior frontal operculum activity, but midbrain activations were maintained. Also notable, both days since surgery as well as errors of commission added as separate covariates completely eliminated any group differences observed in the no covariates analysis. At this stage of the research it is difficult to determine the relationship between each of these variables and their contribution to cognitive deficits in pre-chemotherapy BC patients. Overall, the addition of suspected confounding variables as covariates in the t-test analysis modifies existing group differences, thereby indicating that they are impacting cognition-related brain activity and should be considered in baseline analyses of pre-chemotherapy patients.

In an attempt to understand the results more thoroughly, analyses contrasting the active tasks with the rest condition were performed and revealed more additional group differences.

Patients showed significantly less widespread brain activation compared to controls when subtracting rest from either of the active tasks (either press for 2back or press for O). (Patients did show significantly more activation than controls in some region, but this was a less substantial finding than the latter.) Such a distinction between groups perhaps suggests that reduced brain activity in patients in both these contrasts is due to increased neural activity at rest, lessened activation during both of the active task blocks or a combination of both. Overall, patients show less of a magnitude of activation between the

rest condition and both of the active task conditions compared to controls. One study applied PET techniques to investigate glucose metabolism at rest in chemotherapy-treated patients compared to non-chemotherapy cancer patients [107]. Patients who had recently received larger doses of chemotherapy revealed lower glucose metabolic rates in bilateral prefrontal cortex, cerebellum, posterior medial regions and limbic lobe compared to healthy controls and patients who had received smaller chemotherapy doses at an earlier time. (There were no differences between Late-low patients compared to those without chemotherapy treatment.) This study indicates that patients treated with chemotherapy may have altered resting states, yet it seems to be transient in nature. The prevailing brain regions uncovered as significant in this PET study are among those found activated at a lesser extent in patients compared to controls in the current paper when rest is subtracted from the active task. Meanwhile there is some overlap in the frontal and limbic regions where patients inversely show larger activity compared to controls, as well as consistently in the thalamus, striatum and brainstem. However, one must consider that the visuospatial task used in this paper had only 2 rest epochs of 24 seconds each. Such a small rest block could mean that participants were not truly at rest, possibly still dwelling on actions related to the preceding active task. Longer rest conditions or resting state fMRI to investigate the default network functioning in BC patients, both pre- and post-treatment are warranted and should be conducted in future studies. Additionally, investigations into the ability of patients to "sustain" active task brain activations compared to controls would be interesting. It is possible that the major finding concerning decreased magnitude of activity between active task and rest seen in patients could be a consequence of difficulties in sustaining active task attention. Patients may potentially be fluctuating between neural engagement during the active task and default brain activity over the course of the active task blocks themselves.

Further statistical exploration of the data included regressions analyses considering factors such as errors of commission, reaction time, percent correct and days since surgery. While it is expected to find within-group variability in any study sample, the nature of heterogeneity revealed in the patient and control samples, respectively, is quite interesting. There were no significant relationships between brain activation and reaction time or days since surgery. However, regression analyses in patients along errors

of commission revealed a positive relationship between the number of erroneous button presses during the task and the amount of neural activity in the insula, cingulate gyrus, hippocampus, parahippocampus, inferior temporal gyrus, thalamus, fusiform as well as brainstem and cerebellum. This is particularly interesting given the small number of commission errors committed by both groups of participants. Patients, who had slower response times than controls also made less of these mistakes, suggesting a compensation of speed for accuracy or a more cautious approach in the patient group. The only other pre-chemotherapy fMRI study of working memory to show significant differences between BC patients and controls also revealed slower reaction time and less accuracy in patients compared to controls. 0. Meanwhile, in the control population, there was increased widespread brain activity as less commission errors were being committed, mainly in frontal and parietal regions. There is little overlap between the activated regions found in patients and controls, again highlighting the differences in neural processing during visuospatial working memory between pre-treatment patients and controls along this variable.

Similarly, when considering percent correct scores, controls revealed an expected relationship between neural activity and performance, with more activation in frontal areas and parahippocampal gyrus related to better performance. Patients did not show this relationship, thereby not revealing within group variability when considering this variable's impact on brain functioning. Therefore, it seems like patients do not show a typical linear relationship between task performance and increased brain activations as patients with task success show the same brain activity as those who committed many errors of omission. These within-sample regression results highlight the importance of including more than just analyses of group differences (such as t-test or factorial analysis) as these may wash out important subtle variations revealed in the individual groups. The opposite relationship between task commission errors and neural activity in patient and control populations indicates different within-group variability. As well, it is interesting to note that patients do not reveal a typical linear relationship between increased brain activity and higher task performance, as seen in controls. Such within-group relationships should additionally be systematically studied since it could yield a better understanding

of post-chemotherapy sequelae and possible pre-disposition factors leading to more serious cognitive impairment.

To our knowledge, this study is the largest prospective fMRI investigation of brain activation patterns during visuospatial working memory of pre-chemotherapy cancer patients compared to individually-matched controls. Possible limitations should be considered. First, the present study was performed on a 1.5T scanner. As shown in a previous study [108], use of a 3T scanner could uncover more activations, particularly in the frontal regions. Second, a smaller number of patients and controls, compared to the total sample, completed the diurnal cortisol and estrogen salivary sampling sessions. This number was restricted in patients due to practical constraints. While not a significant effect, pre-treatment BC patients in this study revealed a flattened diurnal cortisol curve similar to that observed in post-treatment studies [[109], [110]]. As well, it is known that successful working memory is affected by HPA activity [[111]-[113]]. Therefore, future investigations of pre-treatment BC patients should consider cortisol assessments in a larger population. Lastly, it is difficult to separate working memory from other cognitive processes such as impulsivity and attention, which may also be impacted by factors related to BC, pre-chemotherapy. This was controlled as much as possible in the current block design task, but perhaps an event related design could isolate these different processes further.

In conclusion, these results provide preliminary but compelling evidence of neurofunctional differences between BC patients and controls associated with visuospatial working memory, prior to chemotherapy. Additionally, they expose the importance of certain confounding variables, leading to the suggestion that these should be studied as primary variables of interest in future research. Also, the findings indicate a polar neurofunctional relationship between task performance and brain activity in patients compared to controls, as well as potential differences in the magnitude of brain activity between active task engagement and rest. These results have significant implications for future cancer and chemotherapy cognitive research, concerning both study designs and analyses. Understanding cancer patients prior to chemotherapy, and the contribution of confounding variables on baseline differences will lead to a more precise understanding of cognitive impairments related to chemotherapy-treatment itself. It will also assist with

identifying pre-chemotherapy cognitive vulnerability, particularly in working memory, an essential capacity in this challenging time in their life. A new cancer diagnosis leads to many challenges for patients, both biological (cytokine activity, anesthesia) and personal (stress, anxiety, depression) which deserve to be systematically studied.

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Table A **Demographic and Clinical Data**

Factors	Patients (n=23)	Controls (n=23)
Age	51.5 (8.47)	50.4 (8.82)
Estimated IQ	105.8 (9.05)	109.22 (8.43)
Education Level		
High School	2	3
College	10	11
Bachelors	6	3
Masters	3	6
PhD	1	0
Marital Status		
Married / Common Law	19	14
Single	0	3
Separated / Divorced	2	6
Widowed	2	0
Days since surgery	47.6 (Max 71, Min 28)	---
Cancer stage		
1	4	---
2a	10	---
2b	5	---
3a	4	---
Menopausal status		
Menstruating	8	9
Perimenopausal	3	2
Postmenopausal	12	12
BDI *	8.3 (8.92)	3.78 (3.06)
BAI *	7.81 (7.25)	4.13 (4.35)
Neuropsychological Domain scores		
1. Verbal memory	-0.24 (1.19)	---
2. Processing speed	-0.11 (0.91)	---
3. Attention	-0.26 (0.86)	---
4. Visual memory	0.22 (0.78)	---
5. Reasoning	-0.35 (1.48)	---
6. Verbal short-term memory	-0.17 (0.65)	---

* = Significant group difference (2 way independent t-test, $p < 0.05$)
Age, Estimated IQ, BDI, BAI, Domain scores = Mean (Stdev.); Educational level, Marital Status, Menopausal status = n; Days since surgery: # of days between surgery and baseline scan; Domain scores = standardized to controls.
Abbreviations: IQ= Intelligence quotient, BDI = Beck Depression Inventory, BAI = Beck Anxiety Inventory

Table B **Significant t-test results comparing patients and controls for the 2back-0 contrast**

Patients > Controls

Condition	Region	Coordinate	ROI P_{FWE}	K	T value	Z value
No covariates	L Inferior frontal operculum	-36 6 16	0.050	6	4.29	3.90
	R Midbrain	4 -20 -2	0.035	14	3.56	3.32
Cortisol	L Thalamus	-4 -20 0	0.047	4	3.81	3.33
Metacognition	None					
RXN time Press for 2back	L Insula	-32 6 18	0.068	13	4.59	4.12*
	R Superior occipital gyrus	24 -86 16	0.044	23	3.99	3.66
	R Thalamus	4 -20 -2	0.034	9	3.90	3.59
	L Thalamus	-4 -10 -4	0.027	19	3.40	3.18
Days since surgery	None					
BDI	R Midbrain (red nucleus)	6 -20 -6	0.004	13	3.67	3.41
BAI	L Insula	-32 6 18	0.052	26	4.93	4.37*
		-40 10 14			4.00	3.67
	R Lateral globus pallidus	18 2 -6	0.016	8	3.76	3.48
Errors of Commission	None					

Tables show significant ROI investigation at threshold 0.001 uncorrected, and P_{FWE} for multiple comparisons. N= 23 patients and 23 controls, except cortisol with 12 patients and 15 controls. (R = right, L = left, K = cluster size) ; * indicates significance at Bonferroni adjustment for multiple comparisons.

Table C **Significant flexible factorial results comparing patients and controls for the 2back-rest and 0-rest contrasts**

Patients > Controls

Condition	Region	P_{FWE}	Coordinate	T value	Z value
O-rest	L Inferior frontal gyrus	0.001	-58 12 26	5.87	5.02
		0.019	-56 18 0	5.93	5.05
	L Medial superior frontal gyrus	0.002	-6 32 64	8.37	6.44
	L Precentral gyrus	0.001	-58 6 20	6.77	5.58
			-58 8 10	6.35	5.32
			-54 6 16	5.52	4.78
	R Postcentral gyrus	0.007	2 -42 76	6.79	5.59
		0.012	68 -30 18	6.15	5.19
	L Insula	0.000	-40 2 -12	6.49	5.41
			-38 -2 -12	6.21	5.24
	L Inferior frontal operculum	0.001	-58 8 10	6.35	5.32
			-58 12 26	5.87	5.02
			-58 14 6	5.84	4.99
			-58 10 18	5.83	4.99
	L Parahippocampus	0.000	-28 6 -18	6.80	5.59
	L Superior temporal gyrus	0.000	-46 4 -14	6.74	5.56
			-32 8 -18	6.63	5.49
			-36 2 -22	5.70	4.91
		0.002	-66 -22 10	7.50	5.99
	L Claustrum	0.000	-34 -10 -8	5.90	5.03
	L Lentiform nucleus	0.000	-24 -18 0	6.61	5.48
			-26 -6 -2	5.79	4.97
	L Putamen	0.000	-28 -10 -4	7.05	5.74
			-32 -6 -6	7.03	5.72
	L Thalamus	0.000	-22 -22 -2	6.87	5.63
	L Brainstem	0.013	-4 -32 -10	6.00	5.10
	R Brainstem	0.018	4 -8 -20	5.40	4.71
2back-rest	L Inferior frontal gyrus	0.000	-32 8 -16	7.50	5.99
		0.003	-54 18 4	7.22	5.83
	L Medial frontal gyrus	0.003	-8 36 62	8.06	6.28
	L Precentral gyrus	0.015	-58 6 10	5.79	4.96
	L Insula	0.000	-42 4 -10	7.57	6.03
			-38 0 -10	7.46	5.96
			-34 8 -10	5.84	5.00
	L Hippocampus	0.000	-26 -28 -8	6.64	5.50
			-30 -8 -16	5.83	4.99
			-26 -24 -14	5.82	4.98
			-30 -14 -20	5.54	4.73
	L Parahippocampus	0.000	-28 -6 -22	6.09	5.16
			-28 6 -18	8.34	6.43
			-14 -26 -18	6.45	5.38
			-28 -4 -16	5.59	4.83
			-32 -10 -24	5.44	4.73
		0.020	-18 -44 -2	6.09	5.16
	L Superior temporal gyrus	0.000	-36 2 -22	6.63	5.49
		0.012	38 6 -18	5.78	4.96
	R Supramarginal gyrus	0.018	68 -26 18	6.31	5.30
	R Angular gyrus	0.015	40 -70 50	6.46	5.39

	L Lentiform nucleus	0.000	-26 -6 -2	7.52	6.00
			-26 -12 -2	7.32	5.89
			-22 -12 -4	6.86	5.63
			-20 -10 6	6.60	5.47
	L Putamen	0.000	-32 -6 -6	7.99	6.25
			-30 -2 -6	6.86	5.63
	L Thalamus	0.000	-20 -18 0	7.48	5.98
			-8 0 2	7.47	5.97
			-22 -24 -2	7.09	5.76
			0 -10 0	6.69	5.53
	R Thalamus	0.000	2 -14 2	6.84	5.62
	R Lingual gyrus	0.013	24 -90 -10	6.02	5.12
	R Precuneus	0.003	4 -46 74	7.53	6.00
		0.011	10 -72 60	6.27	5.27
	L Brainstem	0.000	-4 -32 -10	6.33	5.31
	R Brainstem	0.001	6 -8 -20	6.45	5.39

Patients < Controls

Condition	Region	P_{FWE}	Coord.	T value	Z value
O-rest	R Middle frontal gyrus	0.000	30 34 -2	11.83	Inf.
	L Medial frontal gyrus	0.000	-16 38 24	13.38	Inf.
	L Superior frontal gyrus	0.000	-20 22 40	11.79	Inf.
	R Precentral	0.004	52 2 24	5.88	5.02
	L Cingulate gyrus	0.000	-16 -14 36	14.41	Inf.
			-20 -18 40	12.68	Inf.
			-16 2 40	11.86	Inf.
			-16 0 34	11.72	7.84
	L Anterior cingulate gyrus	0.000	-18 34 22	12.85	Inf.
	L Insula	0.000	-26 28 2	12.82	Inf.
			-36 6 16	15.68	Inf.
			-40 10 14	12.28	Inf.
	L Inferior temporal gyrus	0.000	-50 -22 -18	6.68	5.52
	R Middle temporal pole	0.007	44 18 -42	7.68	6.08
	R Inferior parietal lobule	0.001	40 -44 26	6.77	5.57
	R Supramarginal gyrus	0.001	48 -42 30	7.16	5.80
	L Angular gyrus	0.004	-40 -68 32	6.02	5.12
	L Lentiform nucleus	0.008	-14 0 -6	7.06	5.74
	R Putamen	0.000	26 22 2	12.47	Inf.
	L Middle occipital gyrus	0.019	-20 -88 -14	8.75	6.63
	L Precuneus	0.001	-14 -60 34	8.16	6.34
			-16 -54 36	6.99	5.70
			-20 -58 34	6.28	5.28
			-14 -50 42	5.86	5.01
			-12 -46 44	5.49	4.76
	R Precuneus	0.002	20 -54 34	7.87	6.18
	L Cuneus	0.001	-10 -68 26	5.67	4.89
	R Cuneus	0.016	18 -88 16	5.86	5.01
	L Cerebellar tonsil	0.018	-40 -52 -44	6.86	5.63
2back-rest	R Middle frontal gyrus	0.000	40 34 -4	7.93	6.22
	L Medial frontal gyrus	0.000	-16 38 24	13.13	Inf.
	L Superior medial frontal gyrus	0.002	0 50 50	6.96	5.69

	L Cingulate gyrus	0.000	-16 -14 36	13.11	Inf.
			-20 -18 40	11.93	Inf.
	R Cingulate gyrus	0.000	18 2 36	12.51	Inf.
	L Anterior cingulate gyrus	0.000	-18 34 22	12.98	Inf.
			-18 34 28	12.32	Inf.
	L Insula	0.000	-26 28 2	12.50	Inf.
			-26 24 14	11.88	Inf.
	R Insula	0.000	46 -4 14	7.79	6.14
			42 -4 16	6.78	5.56
			34 -2 16	5.63	4.86
	L Inferior temporal gyrus	0.000	-46 -28 -12	11.44	7.75
	R Inferior temporal gyrus	0.000	42 -34 -10	9.59	7.01
	L Middle temporal gyrus	0.000	-42 -70 28	6.59	5.46
			-48 -74 28	5.96	5.08
			-46 -78 26	5.76	4.94
		0.019	-46 -52 -4	5.97	5.08
	R Middle temporal pole	0.016	42 20 -42	6.40	5.35
	L Superior temporal gyrus	0.000	-36 -58 28	6.34	5.32
	R Inferior parietal lobule	0.008	38 -48 26	5.50	4.77
	L Superior parietal lobule	0.018	-24 -56 62	5.63	4.86
	R Supramarginal gyrus	0.008	48 -42 28	5.72	4.92
	L Angular gyrus	0.000	-40 -68 32	6.70	5.53
	L Precuneus	0.000	-14 -60 34	8.04	6.28
			-20 -58 34	6.75	5.56
			-10 -68 26	6.49	5.41
			-8 -72 24	5.51	4.78
			-4 -66 26	5.32	4.65
	R Precuneus	0.004	20 -54 34	7.39	5.93
	L Cuneus	0.000	-12 -76 22	6.21	5.24

Tables show significant activations at $P_{FWE} = 0.05$. N= 23 patients and 23 controls, except cortisol with 12 patients and 15 controls. (R = right, L = left)

Table D **Significant regression results for errors of commission, reaction times and days since surgery.**

Patients

Condition	Region	ROI P_{FWE}	K	Coordinate	T value	Z value
Errors of commission +	L Supplemental motor	0.038	34	-12 -4 74	4.63	3.80
		0.058	14	-6 20 46	4.11	3.48
				-2 -8 76	4.05	3.44
	L Precentral	0.038	82	-58 -2 -12	5.40	4.23*
				-56 13 10	4.01	3.42
				-60 -4 22	3.95	3.36
	L Anterior cingulate	0.053	14	-8 24 24	4.70	3.84
	R Posterior cingulate	0.017	50	12 -58 4	4.83	3.92
	L Insula	0.030	55	-26 24 -4	5.81	4.44*
				-26 20 6	5.27	4.16*
	L Hippocampus	0.008	108	-32 -20 -14	4.51	3.73
				-20 -16 -16	4.00	3.41
				-36 -30 -12	3.78	3.26
	R Hippocampus	0.018	56	32 -14 -14	4.28	3.59
				34 -6 -20	4.00	3.41
				34 -24 -12	3.98	3.39
	L Parahippocampus	0.041	41	-34 -50 -6	4.18	3.53
	R Parahippocampus	0.010	100	16 -26 -10	5.31	4.18*
				16 -26 -14	5.20	4.12*
				20 -30 -4	4.86	3.94
				26 -36 -4	4.40	3.66
				22 -36 -6	4.24	3.56
				22 -40 -4	4.16	3.14
				32 -38 -6	3.59	3.14
	L Inferior temporal	0.024	94	-40 -36 -14	4.37	3.64
	R Inferior temporal	0.002	337	46 -38 -18	5.05	4.04*
				48 -52 -10	4.57	3.77
				48 -44 -12	4.37	3.65
	L Fusiform	0.036	61	-32 -54 -6	4.45	3.69
				-38 -28 -18	4.15	3.51
				-44 -38 -18	3.66	3.18
	R Fusiform	0.006	211	34 -70 -18	4.74	3.87
				34 -72 -14	4.68	3.83
				30 -72 -16	4.58	3.77
	L Supramarginal	0.019	56	-54 -38 26	5.35	4.20*
	L Thalamus	0.001	318	-12 -22 6	5.91	4.49*
				-2 -14 14	5.31	4.18*
				-6 -8 2	4.09	3.47
	R Thalamus	0.000	386	14 -16 2	4.99	4.01*
				18 -24 -2	4.67	3.82
				2 -14 12	4.62	3.79
	L Inferior occipital	0.021	41	-36 -74 -12	4.18	3.53
	R Middle occipital	0.047	41	52 -62 -10	4.13	3.50
	L Lingual	0.013	118	-12 -54 -4	4.24	3.57
				-28 -56 -6	4.17	3.52
				-20 -56 -4	3.79	3.27
	R Lingual	0.001	368	12 -62 2	4.89	3.95
				20 -44 -2	4.49	3.72
				12 -54 -4	3.97	3.39
		0.028	67	8 -86 -12	4.27	3.59
	L brainstem	0.006	190	0 -30 -18	4.29	3.60
				-14 -24 -2	4.71	3.85
				-10 -24 -16	4.13	3.49
	R Brainstem	0.002	322	14 -26 -12	5.38	4.22*
				10 -24 -16	5.28	4.14*
				16 -26 -5	5.14	4.09*
				20 -30 -44	4.86	3.94
				16 -18 -2	4.26	3.57
	L Anterior cerebellum culmen	0.018	106	-34 -40 -32	4.09	3.47
				-34 -56 -26	3.99	3.40

	R Anterior cerebellum culmen	0.036	57	36 -38 -34	4.13	3.49
				28 -36 -36	4.12	3.49
	L Posterior cerebellum declive	0.039	37	-32 -58 -24	4.17	3.52
				-24 -62 -22	3.59	3.13
	R Posterior cerebellum declive	0.004	214	38 -62 -26	4.85	3.93
				32 -68 -20	4.55	3.75
Errors of commission -	R Anterior cingulate	0.050	19	12 3610	4.09	3.47
				18 38 16	3.60	3.14
	R Corpus callosum	0.020	74	14 34 10	4.38	3.65
Reaction times +	No					
Reaction times -	No					
Percentage correct +	No					
Percentage correct -	No					
Days surgery +	No					
Days surgery -	No					

Controls

Condition	Region	ROI P_{FWE}	K	Coordinate	T value	Z value
Errors of commission +	No					
Errors of commission -	L Inferior frontal orbital	0.015	72	-24 28 -20	5.36	4.21*
	L Medial frontal	0.055	54	-14 -4 54	5.74	4.40*
	R Superior frontal orbital	0.007	103	20 28 -20	5.65	4.36*
	L Postcentral	0.000	507	-52 -20 54	5.23	4.14*
				-34 -40 68	4.69	3.83
				-42 -36 52	4.64	3.81
				-32 -38 64	4.58	3.77
				-35 -36 62	4.40	3.66
				-38 -32 56	4.15	3.51
				-34 -38 58	4.09	3.47
				-32 -40 52	4.03	3.43
	R Postcentral	0.002	270	48 -32 50	5.77	4.42*
				26 -48 56	4.98	4.00*
				38 -36 48	4.45	3.70
	R Anterior cingulate	0.009	105	2 12 -8	4.65	3.81
				6 20 -2	4.56	3.76
	L Caudate	0.001	203	-14 20 -8	5.55	4.31*
				-10 12 -12	4.81	3.91
	R Caudate	0.006	89	6 14 -4	4.68	3.83
	L Middle temporal	0.046	66	-46 -82 20	4.10	3.48
	R Superior temporal pole	0.036	27	44 24 -24	4.02	3.42
	L Inferior parietal	0.003	200	-42 -40 50	4.48	3.71
				-40 -46 54	4.33	3.52
				-44 -54 60	3.84	3.30
				-38 -46 44	3.72	3.22
	R Inferior parietal	0.000	393	46 -36 52	5.93	4.50*
				36 -44 46	5.64	4.35*
				42 -38 50	5.43	4.24*
				32 -46 50	5.37	4.21*
				56 -30 52	5.02	4.03*
	L Middle occipital	0.000	393	-20 -94 4	4.97	3.99*
				-40 -92 12	4.49	3.72
				-32 -98 14	4.45	3.69
				-44 -84 10	4.31	3.61
				-18 -102 10	4.30	3.60
				-28 -96 10	4.27	3.59
				-18 -100 4	4.24	3.56
				-44 -86 14	4.22	3.55
	L Middle occipital	0.013	118	-24 -68 36	4.89	3.95
				-30 -74 22	3.68	3.20
	L Precuneus	0.013	118	-24 -68 34	5.01	4.02*
	R Precuneus	0.012	119	6 -70 60	5.26	4.16*
Reaction times +	No					
Reaction times -	No					

Percentage correct +	L Inferior frontal	0.041	70	-40 20 -4	5.34	4.20*
	L Superior orbital frontal	0.028	33	-12 50 -20	4.61	3.79
	L Medial frontal	0.011	51	-8 48 -8	4.06	3.45
	L Parahippocampus	0.021	42	-14 4 22	5.02	4.02*
Percentage correct -	No					

Table shows significant ROI investigations at threshold 0.001 uncorrected, and P_{FWE} for multiple comparisons. N= 23 patients, 23 controls, except cortisol at 12 patients / 15 controls. (R = right, L = left; (+) = positive, (-) = negative) ; * indicates significance at Bonferroni adjustment for multiple comparisons.

Figure 1- **Diurnal cortisol rhythms for breast cancer patients and matched controls**

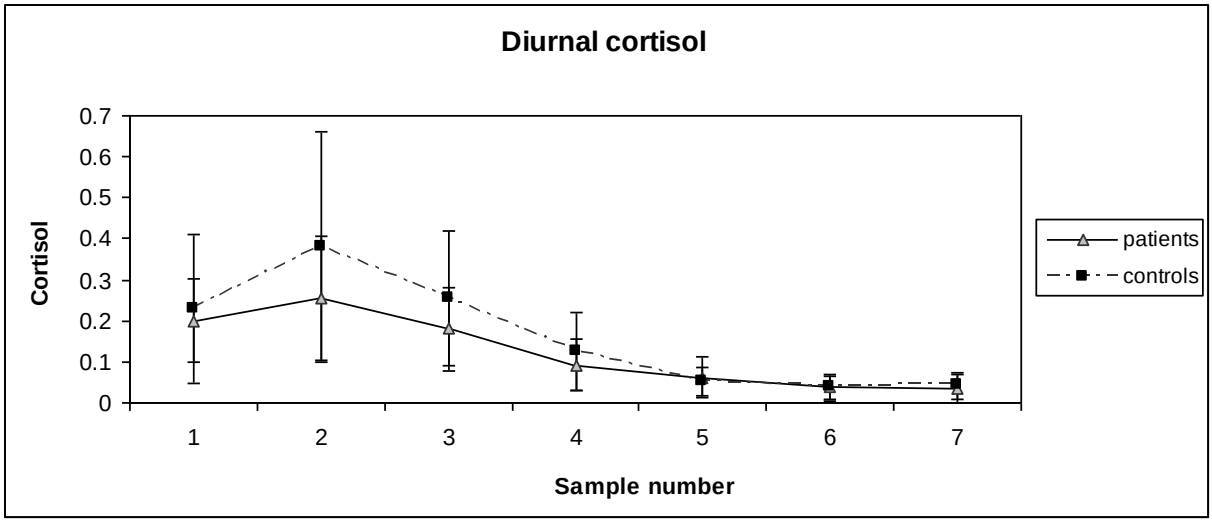
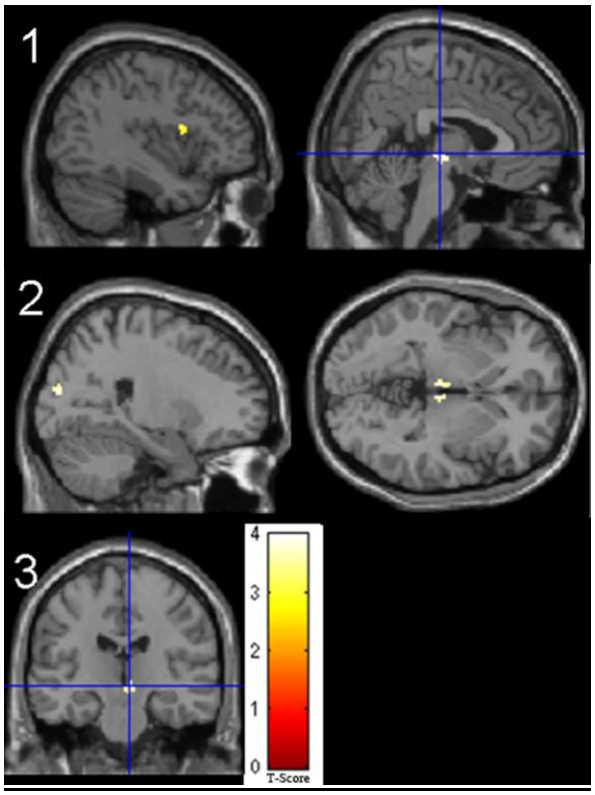
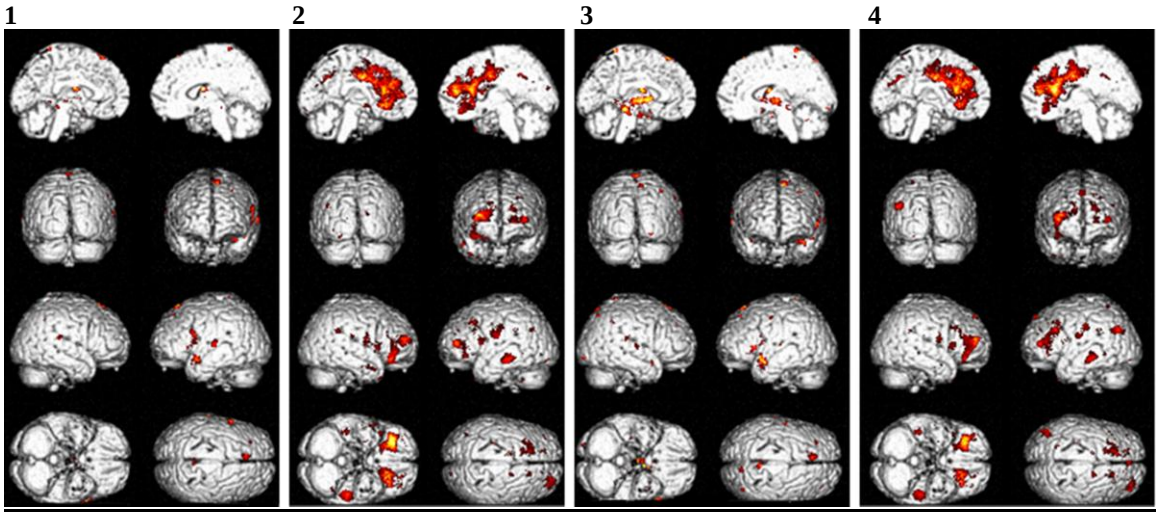


Figure 2- **Significant t-test results for patients > controls for the 2back-0 contrast**



1. No covariate (left frontal operculum, right midbrain);
2. Reaction time as covariate (bilateral thalamus, right superior occipital gyrus);
3. BDI as covariate (right red nucleus)

Figure 3- **Significant flexible factorial results for 0-rest and 2back-rest contrasts**



1) Patients > Controls 0-rest contrast;
2) Patients < Controls 0-rest contrast;
3) Patients > Controls 2back-rest contrast;
4) Patients < Controls 2back-rest contrast.

Supplementary material

Table A suppl. Significant fixed-effects for patients and controls for the 2back-0 contrast

Condition	P_{FWE}	K	Region	Coord.	T value	Z value
Patients	0.000	369	L Inferior frontal gyrus	-46 6 34	6.62	5.48
				-50 12 20	5.85	5.01
				-46 4 26	5.68	4.89
				-50 10 24	5.56	4.81
				-42 8 22	5.51	4.78
				-52 16 24	5.44	4.73
			L Middle frontal gyrus	-46 26 26	7.00	5.71
			L Insula	-46 10 14	5.85	5.01
	0.000	400	R Inferior frontal trigeminal gyrus	44 28 20	6.34	5.32
			R Middle frontal gyrus	46 38 24	7.51	5.99
				44 34 26	6.77	5.57
				48 34 38	6.62	5.49
				42 32 22	6.32	5.30
				46 28 24	5.77	4.95
				46 22 38	5.47	4.75
				48 18 14	5.45	4.74
	0.009	23	R Inferior frontal gyrus	50 18 2	5.68	4.89
	0.000	441	L Middle frontal gyrus	-28 4 54	8.15	6.33
				-30 2 50	7.85	6.17
				-24 2 56	7.42	5.94
				-26 4 60	7.34	5.90
			L Precentral gyrus	-40 -2 46	5.23	4.59
	0.000	219	R Middle frontal gyrus	32 60 8	6.56	5.45
				32 52 14	5.65	4.87
	0.000	333	R Middle frontal gyrus	30 22 52	6.21	5.24
				38 22 50	6.03	5.12
				32 16 52	5.98	5.09
				24 12 52	5.83	4.99
			R Superior frontal gyrus	32 10 56	6.53	5.43
				28 16 54	5.73	4.92
				26 0 52	5.71	4.91
	0.000	850	R Medial frontal gyrus	6 24 46	8.88	6.69
				4 22 50	8.82	6.66
			L Medial frontal gyrus	-4 22 48	8.50	6.50
			R Superior medial frontal gyrus	6 30 44	8.44	6.47
	0.022	8	L Inferior frontal gyrus	-30 22 -4	5.42	4.72
	0.030	4	R Insula	30 24 0	5.39	4.69
	0.003	47	R Inferior temporal gyrus	58 -46 -16	5.14	4.53
			R Middle temporal gyrus	60 -44 -8	5.55	4.80
	0.023	7	R Inferior temporal gyrus	54 -48 -16	5.25	4.60
	0.000	1367	R Inferior parietal lobule	36 -64 42	7.81	6.15
				40 -64 46	7.75	6.12
				40 -62 38	7.20	5.82
				36 -54 40	6.89	5.64
				42 -42 36	6.19	5.22
				46 -50 34	6.14	5.19
				42 -56 38	6.07	5.14
			L Superior parietal lobule	-12 -66 58	6.58	5.46
				-20 -72 56	6.24	5.26
			R Superior parietal lobule	36 -68 44	7.71	6.10
				14 -74 58	6.82	5.60
			R Supramarginal gyrus	40 -50 38	6.99	5.70
				40 -54 42	6.35	5.32
				48 -44 32	5.97	5.08
				50 -48 32	5.29	4.63
			R Superior occipital	30 -64 40	7.47	5.97
			L Precuneus	-14 -72 58	6.96	5.68
				0 -60 60	5.25	4.60

			R Precuneus	8 -66 58	7.34	5.90
				30 -68 42	7.22	5.84
				10 -70 58	7.21	5.83
	0.000	112	R Inferior parietal gyrus	44 -44 56	6.14	5.19
	0.001	80	L Supramarginal gyrus	-38 -46 36	6.24	5.25
	0.030	4	R cuneus	26 -84 20	5.25	4.60
Controls	0.000	7600	L Inferior frontal gyrus	-54 12 18	6.99	5.70
				-58 18 28	6.94	5.67
				-50 10 24	6.76	5.57
			L Precentral	-48 8 34	7.17	5.81
				-42 6 34	6.82	5.60
			L Middle frontal gyrus	-42 24 30	9.90	7.14
				-28 4 54	9.25	6.86
				-24 4 56	8.88	6.69
				-54 20 36	7.19	5.81
			R Middle frontal gyrus	44 38 24	10.92	7.56
				44 30 35	9.15	6.81
				44 24 35	8.65	6.58
				35 8 54	8.48	6.50
				35 50 10	8.24	6.37
				42 18 35	8.23	6.37
				50 20 38	8.13	6.32
				38 30 30	7.95	6.23
				42 32 30	7.87	6.18
				30 8 58	7.82	6.16
				30 6 52	7.65	6.07
				28 22 52	7.59	6.04
				50 14 46	7.24	5.84
				32 16 52	7.07	5.75
				40 20 48	6.59	5.47
				22 10 66	6.56	5.45
			L Medial frontal gyrus	-4 20 48	9.38	6.92
			R Medial frontal gyrus	6 22 50	10.56	7.42
				8 24 46	10.46	7.38
			R Superior frontal gyrus	24 8 54	6.98	5.70
				26 0 52	6.56	5.45
	0.001	70	L Insula	-34 22 0	6.15	5.19
	0.000	5761	L Inferior parietal lobule	-38 -46 42	9.59	7.01
				-40 -42 42	8.46	6.48
				-34 -52 40	8.00	6.25
				-40 -46 50	6.55	5.45
			R Inferior parietal lobule	38 -64 40	8.64	6.57
				36 -68 42	8.55	6.53
				36 -64 46	8.06	6.28
				44 -48 54	7.77	6.13
				36 -52 40	7.70	6.10
				48 -46 56	7.65	6.07
				40 -54 42	7.13	5.78
			L Superior parietal lobule	-22 -70 56	9.23	6.85
				-16 -68 58	9.12	6.80
				-12 -72 56	9.05	6.77
				-16 -72 54	8.83	6.66
			R Superior parietal lobule	32 -54 42	6.98	5.70
				30 -64 56	6.52	5.43
				22 -72 58	6.33	5.31
			L Supramarginal gyrus	-35 -48 38	9.34	6.90
			R Supramarginal gyrus	38 -48 38	8.36	6.43
			R Middle occipital gyrus	36 -68 32	7.30	5.88
				36 -74 32	6.77	5.58
				30 -70 30	6.45	5.39
			L Precuneus	-12 -66 58	8.65	6.58
				0 -50 60	7.07	5.75
				-26 -72 40	6.85	5.62
				-30 -70 42	6.37	5.34

			R Precuneus	30 -68 42	8.52	6.51
				30 -64 40	8.51	6.51
				8 -66 58	7.74	6.12
				12 -74 54	7.70	6.10
				34 -76 38	7.17	5.81

Tables show significant activations at $P_{FWE} = 0.05$. N= 23 patients and 23 controls, except cortisol with 12 patients and 15 controls. (R = right, L = left, K = cluster size)

Chapter 5

Title: Pre-chemotherapy differences in response inhibition in breast cancer patients compared to controls: An fMRI study.

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Abstract

Introduction

Cognitive impairment is a side-effect reported after chemotherapy. A weakness of many studies is the lack of pre-chemotherapy data, essential to unravel treatment-related effects and repercussions of disease, stress and/or surgery. The current study examines neurofunctional differences related to response inhibition between breast cancer (BC) patients and controls, prior to chemotherapy.

Methods

Twenty-three female early-stage BC patients, scanned after surgery but before chemotherapy, were sex-, age- and education-matched to non-cancer controls. fMRI data collected during performance of a Go-Nogo task were analyzed by whole brain and region of interest (ROI) group comparisons. Reaction times, error rates, neuropsychological tests, hospital records and salivary biomarkers were also analysed.

Results

There were no significant group differences concerning neuropsychological tests, estrogen, cortisol, or fMRI task reaction times and error rates. Significant differences between groups were observed for the fMRI data, depending on the type of analysis performed. Most consistent results were widespread attenuated activation in the BC patients compared to controls. Areas affected included cerebellum, cingulate and middle frontal gyrus. Considerable variability in patients' activations compared to controls was also revealed.

Conclusions

This is one of the first multifaceted imaging studies to focus on pre-chemotherapy neural processing differences between BC patients and well matched controls. Results show pre-treatment neurophysiological group differences, highlighting the need to better understand confounding variables and their effects on response inhibition before considering post-treatment effects.

Keywords:
Chemotherapy, breast cancer, pre-treatment cognitive impairment, functional magnetic imaging, response inhibition.

Highlights

- Prior to chemotherapy, BC patients show reduced left cerebellar activity compared with controls during response inhibition.
- Confounding variables, such as days since surgery, significantly impact neurofunctional differences between patients and controls.
- Contrasts of active task and rest conditions reveal decreased patient activations compared to controls.
- The patient group shows considerable sample heterogeneity in neural task activity compared to controls.
- Results emphasize the need to better understand confounding variables and their effects on executive functioning before considering post-chemotherapy effects.

1. Introduction

The study of cognitive deficits, often reported in chemotherapy-treated cancer populations, is an increasingly relevant research topic. Such dysfunctions are particularly evident in breast cancer (BC) patients receiving adjuvant chemotherapy treatment. With the advent of better interventions, a larger number of BC patients are achieving long-term survival, but the associated cognitive deficits reported can potentially persist for many years considerably affecting quality of life. Cancer patients have coined the terms "chemofog" and "chemobrain" to refer to such impairments that can manifest during and after treatment. The prevalence of chemotherapy-related cognitive deficits is highly variable with a range of 17% to 75% reported across studies 0. Neuropsychological studies of "chemofog" have generally confirmed that patients who received chemotherapy treatment perform more poorly on neurocognitive tests compared to non-exposed controls 0-0. Additionally, neuroimaging studies have revealed both structural and functional differences between BC patients and controls, even when variations observed in performance scores between groups are very subtle [0-0]. Overall, both neuropsychological and neuroimaging studies indicate sequelae in processing speed and executive functioning, including working memory. These cognitive processes require the integrity of the frontal brain regions, which have been shown to be affected in both structural and functional neuroimaging studies of chemofog.

One major drawback in the chemofog literature is the minimal investigation into pre-treatment comparisons between BC patients and appropriately matched healthy controls. Longitudinal studies require an understanding of not only the cognitive status of BC patients post-chemotherapy, but also their cognitive abilities prior to chemotherapy. If BC patients are at a different cognitive level than controls at baseline, it is difficult to assess the contribution of the actual chemotherapy as the sole source of cognitive impairment. Many factors could contribute to these deficits, including assessment tasks administered, nature of the control group, cross-sectional versus prospective studies, stress of a cancer diagnosis, and effects of anaesthesia. One study [0] systematically investigated verbal working memory in 10 BC patients prior to chemotherapy-treatment compared to 9 controls using functional magnetic resonance imaging (fMRI) techniques.

The authors indicated pre-treatment differences between the groups, with BC patients showing slower reaction times in the high-demand condition of the task as well as less accuracy in their responses. Patients also revealed larger activation in the right inferior frontal gyrus following increased cognitive demand, as well as additional components of attention/working memory circuitry in both hemispheres compared to controls. This study [0] highlights the importance of obtaining baseline assessments of cancer patients before treatment. These results suggest the existence of pre-treatment cognitive problems, yet the causes are still to be uncovered and larger sample sizes are required to confirm such deficits. Without appropriate baseline testing and scans, the effects of the disease itself, or other potential confounds could be mistaken for an effect of treatment.

The major goal of the current study was to investigate pre-treatment neurophysiological differences between BC patients and well-matched healthy controls. This was accomplished by examining an important component of executive functioning, response inhibition, that may contribute to commonly reported symptoms of chemofog. Response inhibition requires both the integrity of the prefrontal cortex, as well as its connections to the rest of the brain. Deficits in response inhibition have been revealed in populations showing frontal damage [[149]] as well as populations with frontal hypo-functioning [[150]-[153]]. One PET study of chemofog suggests that there are differences in frontal lobe functioning between chemotherapy-treated patients and matched controls, with patients showing decreased resting state metabolism of the left inferior prefrontal cortex. Additionally, when completing a verbal memory recall task, these patients revealed increased regional perfusion of this region [0]. Whether or not these differences in prefrontal functioning impact response inhibition capacities in chemotherapy-treated patients have not been systematically studied in an imaging setting, however, neuropsychological studies have indicated such impairment [0, 11]. One imaging study of memory [10] suggested increased impulsivity in patients compared to controls as they revealed quicker reaction times when encoding information, as well as lower prefrontal activation.

The studies should incorporate several factors that could contribute to cognitive impairment, possibly present before commencement of chemotherapy or exacerbating its effect. One important factor was the presence of cancer itself. This has been considered

by previous studies [7, 9, 0] who matched their chemotherapy-treated patient group to another cancer group receiving a different treatment. However, this "control" group may simply present other confounds related to differences in treatment. For example, the "other patient" group may receive radiation treatment or anti-estrogen regimens, both of which may contribute to fatigue [[156]], which has also been implicated in cognitive dysfunction in BC patients [0]. Another important reason to consider the presence of cancer is evidence that cancer patients have elevated cytokine levels [2]. These small proteins secreted by the immune system both interact with and modulate the activity of other cells in the body which cause disease, inflammation and/or tissue destruction. In particular, high cytokine activity in the hippocampus has been linked with problems of memory consolidation [0]. These negative cytokine effects can persist for 6-60 months after diagnosis compared to controls, with values being highest post-surgery [2].

This also accentuates the need to consider other possible secondary effects of surgery that may be contributing to baseline cognitive functioning. Indeed there is a suggested cognitive decline linked to general anaesthesia administered during surgery. This is predominantly compelling in elderly patients (60+) as they are more prone to develop long term deficits [[159]-1]. This point is particularly important in BC studies since the majority of patients are middle aged to elderly. One imaging study [7] included a covariate of "days between surgery date and baseline MRI scan" in a comparison between a chemotherapy-treated patient group and another cancer group receiving a different treatment. While the time since surgery reduced the statistical significance, it did not change the results, leading the authors to suggest that this confound variable does not influence grey matter volumes. However, the authors compared two groups who had undergone surgery, both possibly showing the same profile of elevated cytokine levels and cognitive deficits related to general anaesthesia. For these reasons and those mentioned above, factors such as the number of days since surgery were considered in the present paper to help tease out possible effects of surgery in the cancer group compared to healthy controls at pre-chemotherapy testing.

Another factor that was included in the present study was the stress experienced following a BC diagnosis. This was measured by salivary cortisol. Stress, routinely measured in chemofog studies through self-report, highly correlates with complaints of

cognitive difficulties [0, 0, 0, 0, 0, 0, [162], [163]]. With increased levels of stress, there is increased activity of the hypothalamic-pituitary-adrenal (HPA) axis, leading to elevated glucocorticoid (GC) levels in the body (cortisol in humans). Increased levels of GCs (also a consequence of therapeutic GC regimens that are part of the immunosuppressive therapy used in cancer treatment) have been shown to affect brain anatomy [[164]-[166]] as well as memory functions, particularly working memory [[167]]. Regions in the brain, such as the hippocampus, are particularly affected by glucocorticoid-mediated excitotoxicity due to an elevated number of GC receptors. In fact, smaller hippocampal volumes are seen in high stress populations, suggesting potential negative effects of higher circulating GCs [[168]-[170]]. One MRI study has shown transiently smaller volumes in key cognitive regions (such as the hippocampus) in BC survivors treated with chemotherapy compared to those treated with surgery alone. These structural differences were mirrored by lower scores on tests of attention, visual memory, and concentration 1 year after chemotherapy [4]. GCs may also have an indirect role in the development of cognitive deficits post-chemotherapy. Higher levels of circulating GCs during high stress times (ex: a cancer diagnosis and/or treatment) may contribute to increased capillary permeability of the blood brain barrier [[171]] which could lead to increased accessibility of chemotherapy agents to the brain. Therefore, increased GC levels could potentially exacerbate the adverse effects of chemotherapy on the brain. Thus, salivary cortisol, a quantifiable measure of stress, was sampled and statistically considered in the present paper.

Finally, a biological measure sampled in this study was salivary estrogen level, which is important considering the typically older BC population and menopausal stage. Estrogen has long been considered to have a neuroprotective role in the brain, thereby helping to maintain cognitive abilities. While the exact mechanism by which it exerts such protection remains unclear, there is likely an interaction between various neurotransmitter systems, neurotrophic factors as well as nuclear estrogen receptors. These are particularly present in areas such as the hippocampus and amygdala, both implicated in learning and memory [[172]-[174]]. After menopause, women report declining cognitive capacities and show decreased bilateral hippocampal volumes- an effect that has been shown to be minimized by estrogen therapy [[175]].

The current study examined functional neural processing in newly diagnosed female BC patients, prior to chemotherapy, and carefully matched controls completing a response inhibition task in an MRI scanner. The Go Nogo task is a common cognitive paradigm that has successfully been applied to examine inhibitory capacities [[176], [177]]. Neural activity related to response inhibition in healthy populations includes diverse bilateral frontal lobe activations (particularly the inferior prefrontal cortex), bilateral supplementary motor cortices, bilateral parietal cortex and bilateral cerebellum [[178]-[180]]. A variety of speculated baseline confounds associated with cancer populations were considered as covariates in the fMRI analysis, including days since surgery, diurnal cortisol levels, scores on depression and anxiety inventories, and task performance scores. It was hypothesized that the addition of these covariates would modify the analyses with no covariates (as seen in previous studies [7]) and reveal baseline differences between patients and controls. Support for these hypotheses would emphasize the multifaceted nature of the BC population and thus the importance of baseline comparisons when studying chemofog.

2. Methods

The patient sample included 23 early stage breast cancer (BC) patients, recruited through the Ottawa Hospital Regional Cancer Centre (average age: 51 ± 8.5 years, range: 35-64). At testing, these patients had undergone mastectomy or lumpectomy for stages I or II BC and were scheduled to begin a chemotherapy regimen. Inclusion criteria were: female, no previous cancer or cancer treatment, no unstable psychiatric and/or neurological illness, no history of substance abuse, Beck Depression Inventory (BDI-II; [[181]]) scores of < 20 and <15 for Beck Anxiety Inventory (BAI; [[182]]) scores, fluency in English, and minimum grade 8 education. fMRI exclusion criteria included left-handedness, sight problems, claustrophobia, pacemaker, and metal implants. Healthy controls (average age: 49 ± 9 years, range: 30-62) were recruited either through nomination or through posters/website ads and each was sex-, age- and education-

matched to an individual cancer patient. Controls met the above inclusion and exclusion criteria and followed the same research procedure as the index patient.

2.1 Neuropsychological Assessments:

At baseline, each participant completed psychometric tests and MRI/fMRI scanning. The 2 ½ hour baseline psychometric test battery included: social /medical history, self-report questionnaires of physical, emotional, and cognitive functioning, traditional pencil-and-paper neuropsychological tests, and a 30 minute computerized cognitive test (CNS Vital Signs; https://www.cnsvs.com/). This computerized test battery, validated [70] in a broad age range and across clinical and non-clinical populations (incl. cancer patients), has proven sensitivity in monitoring a patient's cognitive status over time. In particular this battery measures: attention, reaction time, working memory, executive function, visual episodic memory, and verbal episodic memory. Raw neuropsychological data were converted to standardized scores based on the means and standard deviations of the healthy control group. Summary scores for several cognitive domains were computed as well as an overall cognitive summary calculated by averaging all of the cognitive scores. Cognitive domains were computed based on both rational and empirical grounds. There was a total of nineteen tests comprised in the neuropsychological battery from which the 6 following cognitive domains were extracted: 1. Verbal Memory (Hopkins Verbal Learning Test-Revised (HVLT-R; [71]) and CNSVS Verbal Memory Index), 2. Processing Speed (WAIS-III [72] Digit-symbol coding, WAIS-III Symbol Search, controlled oral word association[73], CNSVS Processing speed index, CNSVS Executive Functioning index, CNSVS Reaction time index and CNSVS Cognitive Flexibility index), 3. Attention (WAIS-III Digit Span, WAIS-III Letter-number sequencing, Paced Auditory Serial Attention Test (PASAT; [74]), CNSVS Sustained attention index and Trailmaking Test, Part B[75]), 4. Visual Memory (CNSVS Visual Memory index, Brief Visuospatial Memory Test-Revised (BVMT-R; [76]) and CNSVS Working Memory index), 5. Reasoning (CNSVS Reasoning index) and 6. Verbal Short- term Memory (Auditory Consonant Trigrams; [77]). At the baseline scanning session, each participant also

completed the Rosenberg Self-esteem questionnaire (RSE; [78]) and the Questionnaire for Competence and Control (FKK; [79]).

Demographic, neuropsychological and self-esteem data were analyzed by 2-tailed independent t-tests using Statistical Package for the Social Sciences version 18.0 program (SPSS v. 18.0, Chicago, IL). A correlational analysis was also conducted to reveal any relationships between factors.

2.2 Assessment of Biological Markers:

Hospital records for each patient were reviewed to extract data on baseline blood work (hemoglobin, white blood cell, neutrophil). Diurnal salivary sampling was collected from each participant using an at-home sampling kit protocol validated to accurately assess the free fraction of the major stress hormone cortisol [[193]]. Participants were provided with a sampling kit containing 14 cotton salivettes and detailed instructions. These instructions emphasized the requirements for participants to follow a rigid sampling schedule for 2 typical and consecutive weekdays. Cortisol was sampled at 7 times points per day for each of the 2 days at: Awakening, 30 minutes after awakening, 1 hour after awakening (food and drink consumption was permitted after this sample), 10am, 2pm, 6pm and 9pm. This sampling schedule provided a circadian cortisol profile for each participant. This was important as cortisol varies according to circadian rhythms and such cycles have been shown to be affected by high stress and in clinical populations [[194]-85]. Participants were asked to store the completed samples in a freezer until the next appointment. The saliva samples were analyzed for cortisol using ELISA techniques (as described by Salimetrics at salimetrics.com). Intra- and inter-assay variability (CVs) for datapoints (excluding outliers) were less than 10% and 15%, respectively. Computation of the area under the curve from ground (AUCg) was completed in accordance to the formulas described by Pruessner [[199]] in order to analyze salivary cortisol differences. AUCg was computed and 2-tailed independent t-tests were applied using SPSS v. 18.0.

Considering that the BC population is typically older and are either pre-menopausal or in menopause, it was essential to measure baseline estrogen levels. Estrogen sampling was also part of the at-home kits, with participants asked to give pure

spit samples at the 10 am mark for each of the 2 consecutive days. The saliva samples were analyzed for estrodiol using ELISA techniques (according to the procedure described by Salimetrics at salimetrics.com). Intra-assay coefficients of variation on one estrogen measurement / participant were less than 0.43 (CV (%) = 4.03, Max: 19 and Min 0.148). Group differences in estrogen levels were assessed by a 2-tailed independent t-test using SPSS v. 18.0.

2.3 Scanning Sessions:

Brain images were acquired using a 1.5 Tesla Siemens Magnetom Symphony MR scanner. Subjects, lying on their back with their head secured in a standard head holder, were instructed to stay as still as possible while in the scanner. A gradient echo localizer was acquired and used to prescribe a subsequent 3D FLASH (Fast Low Angle Shot, a spoiled gradient sequence) (TR/TE 22/9.2ms, flip angle 30º, field of view (FOV) 256x256 mm scan). Whole brain echo planar fMRI, based on the blood oxygen level dependent (BOLD) effect, was performed using a gradient echo pulse sequence (TR/TE 3000/40ms, flip angle 90º, FOV 250x187.5mm, 64x64 matrix, slice thickness 5mm, 27 axial slices, bandwidth 2430 Hz per pixel).

2.4 fMRI Go Nogo task:

The task was projected through an LCD projector from a computer in the control room, and presented on a rear projection screen placed near the end of the patient table. A mirrored head coil allowed the participant to view the task while lying in the scanner. In order to increase visibility, all lighting in the scanning room was off. Participant responses, in the form of button presses on a response pad, were recorded via a MRI compatible fiber optic device (Lumina LP-400, Cedrus).

The Go Nogo was a block-design task used to measure response inhibition processes while in the scanner. Stimuli were white upper case letters presented in the middle of a solid black background. Fifty percent of the stimuli presented were the letter X while the other 50% were randomly presented from the rest of the alphabet. Participants were instructed to use the response pad with their right hand, and to press the correct key as quickly and accurately as possible, using the right index finger. They were

encouraged not to think about or dwell on previous errors. The scanning session began with an initial rest epoch of 9 seconds in order to allow longitudinal magnetic relaxation (T1 effects) to stabilize. Images collected during this initial rest epoch were excluded in the image analysis. The Go Nogo procedure involved two conditions of target presentations, with the letter stimuli being presented one at a time on the screen for 75ms (interstimulus interval of 925ms). The first condition, 'Press for X', required the participant to press a button when an X appeared on the screen and to refrain from pressing when any other letter appeared. The second condition, 'Press for all letters except X', required the participant to press the button when any letter other than X appeared on the screen and to refrain from pressing for the X. Four 27 second blocks of each condition were presented in a counterbalanced order, starting with 'Press for X'. Each block began with a 3 second instruction screen, followed by the presentation of 24 stimuli, half of which required a response for both conditions. A rest block of 18 seconds followed each of these target condition blocks. During the instructions, the participant viewed either 'Press for X' or 'Press for all letters except X'. During rest epochs, the word 'REST' was presented on the screen, instructing the participant that no motor response was required. After the completed task, all participants were asked to rate the difficulty of the task on a 5-point Likert Scale. These values were used to investigate the metacognitive capacities of each participant.

2.5 fMRI Performance Parameters and Analyses

Errors of commission included any incorrect response following a stimulus (ex: pressing for an X in the 'Press for all letters except X' condition) within 900 ms of stimulus presentation. Omission errors were defined as a failure to respond to a target stimulus within the same time frame. All accurate responses occurring within 900 ms of stimulus presentation were used to calculate mean reaction times for both conditions.

Reaction times for each response in the scanner, along with errors of commission were analyzed and metacognition scores on the fMRI task were considered. Group differences were computed by 2-tailed independent sample t-tests using SPSS v. 18.0. A correlational analysis was also applied to reveal if there was a relationship between the cognitive impression of task difficulty and actual task performance.

2.6 Image Processing

Statistical Parametric Mapping 8 (SPM8) was used to post-process the fMRI data and to perform the statistical analyses specific to these data. The functional images were reconstructed and whole brain images were realigned to correct for motion by employing the procedure of Friston and colleagues [[200]]. Images were then spatially normalized to match the echo planar imaging (EPI) template provided in SPM8. Following spatial normalization, images were smoothed with a 10mm full-width at half-maximum Gaussian filter given the slice thickness of 5mm.

First level analyses were performed for each participant using these images representing the two task conditions. Response inhibition processes were isolated by subtracting the averaged 'Press for X' images from the averaged 'Press for all letters except X' images (condition labelled as "a-x"), creating one image per participant. Two additional contrasts were also computed at the first level: "press for X – rest" (or "x-rest") and "press for all letters except X – rest" (or "a-rest"). Additionally, motion correction was applied as a regressor. These processed within-subject images were then entered into the second level analyses for comparisons between patient and control groups. Second level analyses included 3 different assessments. First, a 2-way independent group t-test of the "a-x" contrast was executed without covariates, and then separate t-tests were performed with suspected confound variables applied as covariates in order to extract their possible individual effects. Second, flexible factorial assessments of both "x-rest" and "a-rest" contrasts were conducted in order to assess if the rest condition itself could drive group differences. Third, regression analyses along days since surgery in the patient group, and diurnal cortisol in both controls and patients, were performed for the "a-x" contrast to assess the level of group heterogeneity in response inhibition caused by these factors.

Whole brain and ROI investigations for both t-test and regression analyses were conducted at a set threshold of $p_{uncorr.} = 0.001$ uncorrected, with a cluster-wise correction at $p_{FWE} = 0.05$ and a set cluster size larger than 10 voxels. Whole brain fMRI, more stringent than ROI analyses, measures activation differences over the entire brain and thus any effect size apparent in small areas will be diluted. ROIs investigated

in this study were selected from several sources: the affected regions reported in previous post-chemotherapy structural and functional studies [4-0, 9, 0], related regions implicated in light of possible confound variable effects such as stress and cytokine activity, and areas with a role in response inhibition [[202]]. Bilateral ROIs extracted included: frontal lobe, precentral gyrus, cingulate gyrus, parahippocampus, hippocampus, insula, thalamus, basal ganglia, temporal lobe, parietal lobe, precuneus, occipital lobe, brainstem and cerebellum. Significant results presented are both unadjusted and adjusted for multiple comparisons. A Bonferroni adjustment with 28 comparisons lowers the alpha to 3.57^{-5} with a significant z-value now of 3.97. Meanwhile, whole brain investigations for flexible factorial analyses were conducted at a set threshold of p= 0.05 corrected, with a cluster-wise correction at $p_{FWE} = 0.05$.

3. Results

3.1 Demographic and Clinical Data

Correlations were computed to investigate any relationships between the demographic and neuropsychological factors. Age negatively correlated with attention ($r(46) = -0.311$, $p= 0.035$), reasoning ($r(46) = -0.418$, $p= 0.004$), and processing speed ($r(46) = -0.531$, $p< 0.001$), with younger participants scoring higher on tests comprising these cognitive domains. The Wechsler test of Adult Reading positively correlated with 5 out of the 6 computed cognitive domains: attention ($r(46) = 0.486$, $p= 0.001$), visual memory ($r(46) = 0.332$, $p= 0.026$), reasoning ($r(46) = 0.295$, $p= 0.049$), verbal short-term memory ($r(46) = 0.459$, $p= 0.002$), and processing speed ($r(46) = 0.447$, $p= 0.002$). Additionally, verbal memory positively correlated with reasoning ($r(46) = 0.353$, $p= 0.016$), attention correlated with visual memory ($r(46) = 0.496$, $p= 0.000$), reasoning ($r(46) = 0.596$, $p= 0.000$) as well as processing speed ($r(46) = 0.551$, $p= 0.000$) and visual memory correlated with processing speed ($r(46) = 0.314$, $p= 0.033$). The Beck Depression Inventory (BDI) revealed a larger mean score in patients (8.3) compared to controls (3.8) ($t(44) = 2.299$, $p= 0.026$). Similarly, the Beck Anxiety Inventory (BAI) showed larger scores in patients (7.8) compared to controls (4.1) ($t(44)= 2.090$, $p=0.042$).

As expected, BDI scores positively correlate with BAI scores ($r(46) = 0.342$, $p= 0.020$). A negative correlation was revealed between BDI scores and the processing speed domain ($r(46) = -0.368$, $p= 0.012$) while a similar negative correlation was shown between BAI scores and verbal short-term memory ($r(46) = -0.362$, $p= 0.013$).

Table A summarizes demographic and clinical characteristics for the BC patients and healthy controls. Estimated IQ was assessed by the Wechsler Adult Reading Task, and no significant group differences between patients and controls were revealed ($t(44) = -1.312$, $p = 0.197$). No significant differences existed between groups concerning menopausal status, with half the sample being post-menopausal. There were no significant group differences in any of the 6 cognitive domains or on self-esteem measures.

3.2 Biological Measures

Patient blood work revealed average values in the normal range: Hemoglobin (HGB): 134 grams/L, White blood cells (WBC): 7 x 10^9/L, Neutrophils: 4.21 x 10^9/L. Patients and controls did not differ significantly on salivary estrogen levels ($t(25) = 0.835$, $p>0.412$) with average values (Std dev) being 6.07 (3.89) and 4.98 (2.89), respectively. Each subject's diurnal cortisol index ranged from 0 to 0.973 ug/dL, mean (Std dev) = 0.144 (0.159). As well, AUCg values of diurnal salivary cortisol ($t(25) = -1.308$, $p > 0.203$) were not significantly different between groups, with average AUCg (Std dev) at 12.7 (5.41) and 16.1 (8.68) respectively, for patients and controls. See Figure 1 for the graph of diurnal cortisol rhythms for all participants.

fMRI task performance data

No group differences were revealed between patients and controls concerning reaction times for the "Press for all letters except X" condition ($t(44) = 1.292$, $p > 0.203$) or for the metacognition ($t(44) = -0.467$, $p > 0.643$) scores. Slower reaction times were observed in patients compared to controls (mean (StD): 394ms (37) : 380 ms (32)). However, a trend was observed for errors of commission ($t(44) = -1.748$, $p> 0.087$), with patients making less commission mistakes than controls (3.4 : 4.8).

3.3 fMRI Task Image Analysis

3.3.1 2-way independent group t-test (a-x)

Independent t-test of the a-x contrast (Whole brain P values, MNI coordinates, ROI P values, region, cluster extent, T and Z values) are presented in Table B, at threshold $P_{crit} < 0.001$ uncorrected with $P_{FWE} = 0.05$ cluster-wise correction. The data was first analysed without covariates. To control for possible confounding variables, the following were individually added as covariates in the analyses: cortisol, metacognition, "Press for all letters except X" condition reaction time, days since surgery, BDI scores, BAI scores and the number of errors of commission (See Figure 2).

Overall, fixed effects analyses for each group during response inhibition revealed larger activity in the left supplementary motor area for patients and in the right cerebellar tonsil for controls. When comparing groups, patients showed reduced activity compared to controls in the left cerebellar tonsil, and this was maintained with cortisol as a covariate. Meanwhile, this cerebellar activity was eliminated with the addition of all other covariates. Days since surgery as a covariate introduced decreased activity in the left cingulate gyrus and middle frontal gyrus in patients compared to controls. Patients did not reveal significantly increased activity compared to controls during this response inhibition task.

3.3.2 Flexible Factorial (x-rest and a-rest)

All flexible factorial results for the x-rest and the a-rest contrasts (Whole brain P values, MNI coordinates, ROI P values, region, T and Z values) are presented in Table C, at threshold $P_{FWE} < 0.05$ with $P_{FWE} = 0.05$ cluster-wise correction. For the x-rest contrast (see Figure 3), patients presented reduced activity in the left precentral gyrus, right superior frontal gyrus, right superior temporal gyrus, right fusiform and right posterior cerebellar tonsil compared to controls. For the a-rest contrast (see Figure 4), patients revealed less activation in the right fusiform, right inferior occipital gyrus, bilateral lingual gyrus, right anterior cerebellar culmen and right posterior cerebellar tonsil compared to controls.

3.3.3 Regression (a-x)

Regression results of the a-x contrast along days since surgery (Whole brain P values, MNI coordinates, ROI P values, region, cluster extent, T and Z values) are presented in Table D, at threshold $P_{crit} < 0.001$ uncorrected with $P_{FWE} = 0.05$ cluster-wise correction. Patients revealed more activity related to response inhibition with more days that had passed between the surgery and scan dates. This increased effect was observed in the left middle frontal gyrus, left supplemental motor area, bilateral cingulate gyrus, left precuneus, left paracentral lobule, left supramarginal gyrus, right superior temporal gyrus, bilateral middle temporal gyrus and right inferior orbitofrontal gyrus. Also, patients showed more neural activation related to response inhibition with larger diurnal cortisol AUCg while controls did not reveal significant within-group variability on this factor. This latter effect in patients was observed in bilateral parahippocampus, bilateral pons and right anterior cerebellar culmen.

4. Discussion

It is becoming widely acknowledged that some BC patients manifest cognitive impairments after chemotherapy treatment. This can seriously affect quality of life. Aside from only a few studies investigating and reporting on existing pre-treatment differences [0, 2], little is still known about the cognitive ability of BC patients prior to chemotherapy. The purpose of the current study was to further this investigation through the examination of neural processing during response inhibition in BC patients and well-matched healthy controls. This was the first fMRI study to investigate response inhibition abilities in BC patients using a Go Nogo task and controlling for multiple variables that may contribute to group differences in cognitive processing prior to chemotherapy.

The results from the present study indeed show that BC patients, prior to chemotherapy, have different patterns of neural activity than controls during a task of response inhibition. The results vary depending on the statistical protocol followed and the covariates included, highlighting the need for rigorous analyses procedures.

More specifically, this study indicates that patients show less neural activity related to response inhibition in the left cerebellar tonsil compared to controls. Previous imaging studies have shown that cerebellar activation is important in impulsivity control [[203], [204], [205]]. Similarly, response inhibition studies have revealed the importance of the left cerebellum in successful stop trials, as part of an important fronto-thalamo-cerebellar pathway which mediates inhibitory control [[206], [207]]. It is suggested that the cerebellum assists in both action preparation and movement inhibition [[208]]. In the current study, the cerebellar difference in activation was maintained when cortisol was added as a covariate, but was rendered non-significant with the addition of all other covariates. Therefore, there is a significant difference in neural activations related to response inhibition in the left cerebellar tonsil between patients and controls, but this activity is significantly influenced by other confounding variables.

Of particular interest are the findings when "days since surgery" was added as a covariate. Not only did this variable remove or removed left cerebellar group differences, but it also modified the results to reveal decreased activations in the left middle frontal gyrus and left cingulate gyrus in patients compared to controls. Activation in the left middle frontal gyrus has been observed in successful inhibition trials, with more activation occurring during more difficult inhibition conditions [[209]]. This region has also been shown to have greater activity during invalid task trials compared to valid trials [[210], 98], which suggests a role in attention reorienting. The cingulate gyrus also has a major role in response inhibition concerning error detection [[207], 99]. In particular, the left middle cingulate region is important in tasks that put an emphasis on response selection, such as the Stroop colour interference task [[213]] and the current Go Nogo task. Overall, the inclusion of days since surgery as a covariate introduces new group differences in activation patterns related to response inhibition, particularly in frontal regions of the brain.

Solely extracting group differences in response inhibition does not completely reveal all discrepancies between BC patients and controls. These groups may also be different in the blocks between the active tasks. Patients revealed reduced activity compared to controls when rest was subtracted from the active task (either press for A or press for X). This reduced brain activity could be due to increased neural activity at rest,

lessened activation during both of the active task blocks or a combination of both. Overall, this could potentially indicate that patients show less of a magnitude of activation between the rest condition and both of the active task conditions compared to controls. One PET study investigated brain glucose metabolism at rest in chemotherapy-treated and no-chemotherapy cancer patients [[214]]. There were 2 sub-groups of chemotherapy patients: those with a more recent and higher exposure to chemotherapy (Early-high) and those with a more distant and lower exposure to chemotherapy (Late-low). Patients from the Early-high group showed lower glucose metabolic rates in bilateral prefrontal cortex, cerebellum, posterior medial regions and limbic lobe compared to non-chemotherapy controls. This pattern was also revealed in comparisons between Early-high group and the Late-low patients. There were no differences between Late-low patients compared to those without chemotherapy treatment. Therefore, chemotherapy-treated patients showed altered resting glucose metabolism soon after treatment completion that seems to be transient in nature, yet no comparisons were conducted between patients and healthy controls. While the prevailing effect was in the prefrontal cortex, the left precentral gyrus, right lingual gyrus, and bilateral cerebellum were also reported as being significantly different in chemotherapy and non-chemotherapy groups. In the current paper, these regions were among those reported as being different at baseline between BC patients compared to controls when subtracting the rest condition from the active task condition (press for X or press for A). Therefore, investigations of default network functioning in BC patients, both pre- and post-treatment, are warranted and should be conducted in future studies. Furthermore, it would be interesting to investigate the ability of patients to "sustain" brain engagement during the active tasks compared to controls. It is possible that the decreased magnitude of activity between active tasks and rest could be a consequence of patients fluctuating between neural engagement and default brain activity over the course of the active task blocks themselves.

While it is expected to find within-group variability in any sample, the patient sample in this study revealed larger variability along certain confound variables compared to controls with regression analyses. It is acknowledged that a patient population will experience more stress compared to the matched control group, yet no

significant group differences were revealed between patients and controls concerning diurnal cortisol reactivity (similar to Kesler and colleagues) [10]. Both studies (current paper; [10]) lacked power with small samples and future diurnal cortisol assessments should be conducted with more patients. Practical constraints in the current study prevented many of the participants from completing salivary sampling. Even so, a graph of the AUCg reveals a "flattened cortisol secretion pattern" for patients, which has been reported in other BC studies [[215], [216]]. Such a flattened pattern of cortisol secretion has been associated with impaired negative feedback of the HPA axis [[217]]. As well, there is patient specific within-group variability concerning brain activation during response inhibition which is associated with diurnal cortisol reactivity. Most notably, increased activity in bilateral parahippocampal regions was associated with increased cortisol reactivity. The parahippocampus is continuous with the cingulate gyrus [[218]] which is implicated in response inhibition due to its role in error monitoring [[207], [212]]. Additionally, the parahippocampus is both an important connecting pathway of the limbic system and is one of the few areas, along with the hippocampus, to contain both mineralocorticoid and glucocorticoid receptors [[218]] making it particularly sensitive to the effects of cortisol.

Days since surgery also contributed to within group variability concerning response inhibition related activations. Patients revealed increased widespread neural activity with longer post-surgical intervals. It is possible that anaesthetic agents [1] and/or post-surgery cytokine increases [2] affected neural activation patterns and that their effects are decreased as time passes. Whether patients are "regaining" neural functioning and/or these regional increases in activity with time are compensatory in nature, it remains that the number of days since surgery contributes to patient variability. The time-dependant nature of these changes may potentially account for why some studies are not finding baseline differences between chemotherapy-treated cancer patients and controls. If a study recruits a patient population who underwent their baseline MRI scan soon after surgery, there may be different neural activation patterns compared to a patient population who was scanned much later. Additionally, while it could be suggested that this is a time dependant effect with post-surgery patients "returning" to a baseline that resembles controls, such recuperation could take much longer than time allotted in

these studies, which is usually about 1 month. It is important to note that in some cases, a complete recuperation might be an erroneous assumption as previous studies have indicated long-term cognitive deficits [1]. In general, these results indicate that there may be neural effects of surgery that could be mis-attributed to chemotherapy in the absence of pre-treatment baseline testing.

This study suggests that there are subtle but important differences in neural functioning related to response inhibition between BC patients and healthy controls, even before chemotherapy. Results also point to some of the factors that might be contributing to these pre-chemotherapy differences. Additionally, the neural activation patterns uncovered during the blocks separating the active task trials are different between patients and controls, which suggest the need for future investigation of the default network and/or sustained active task engagement in these patients pre-treatment. Also, solely conducting group comparison analyses (such as t-test or factorial analysis) washes out important subtle variations revealed in the heterogeneous patient population. Therefore, this paper suggests that regression analyses of patients can help uncover small changes occurring in this group over time (ex: days since surgery) and/or neural activation differences due to subtle variations of other confounds (ex: hormonal fluctuations, such as diurnal cortisol). Overall, while each suspected factor in this study was applied as a covariate in a separate t-test or regression analyses, it is acknowledged that these do not present alone and likely work in conjunction. Each was applied separately in order to investigate their individual influence on the response inhibition activation pattern, as well as to maintain power in the analysis by applying one covariate at a time in a 23 participant per group sample.

To our knowledge, this prospective study is the largest fMRI investigation in BC patients and individually-matched healthy controls. It is also one of the few studies to include cortisol sampling to objectively assess the impact of stress on neural functioning in BC patients. A limitation of this study is the use of a block design rather than an event related design. Block designs limit the separation of brain activity between Go and Nogo trials, and increased regional activity could be either due to increased response preparation or to increased response inhibition. Additionally, one must consider that both

conditions of the Go Nogo task involve some level of response inhibition. However, as it is more difficult to withhold responding to a target (‘Press for all letters except X’) than to respond to that target (‘Press for X’), and the ‘Press for all letters except X’ condition introduces a greater demand on the response inhibition circuitry. This is also the case since the participants are primed to press for X since the first two blocks are ‘Press for X’ and 50% of the letters presented in each block are X. The increased challenge presented by the ‘Press for all letters except X’ was confirmed by more errors of commission and slower reaction times during this condition for both BC patients and controls. As well, the conditions were contrasted as ‘Press for all letters except X’ - ‘Press for X’ and given that both conditions involved the same visual stimuli and the same required motor responses, response inhibition should be as isolated as possible.

In conclusion, these results provide preliminary but compelling evidence of neurofunctional differences between BC patients and controls associated with response inhibition, prior to chemotherapy treatment. This study exposes the importance of certain confound variables, indicating that future studies should investigate these as primary variables of interest. Additionally, patient group heterogeneity, and potential differences at rest between patients and controls should also be examined more closely. These findings have significant implications for the designs and analyses of future cancer and chemotherapy cognition studies. Finally, understanding baseline differences and contributing confound variables will lead to a clearer picture of cognitive impairments related to chemotherapy-treatment itself. Patients with a new cancer diagnosis are faced with many challenges both biological (cytokine activity, anesthesia) and personal (stress, anxiety, depression) which deserve to be systematically studied.

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Table A **Demographic and Clinical Data**

Factors	Patients (n=23)	Controls (n=23)
Age	51.5 (8.47)	50.4 (8.82)
Estimated IQ	105.8 (9.05)	109.22 (8.43)
Education Level		
High School	2	3
College	10	11
Bachelors	6	3
Masters	3	6
PhD	1	0
Marital Status		
Married / Common Law	19	14
Single	0	3
Separated / Divorced	2	6
Widowed	2	0
Days since surgery	47.6 (Max 71, Min 28)	---
Cancer stage		
1	4	---
2a	10	---
2b	5	---
3a	4	---
Menopausal status		
Menstruating	8	9
Perimenopausal	3	2
Postmenopausal	12	12
BDI *	8.3 (8.92)	3.78 (3.06)
BAI *	7.81 (7.25)	4.13 (4.35)
Neuropsychological Domain scores		
1. Verbal memory	-0.24 (1.19)	---
2. Processing speed	-0.11 (0.91)	---
3. Attention	-0.26 (0.86)	---
4. Visual memory	0.22 (0.78)	---
5. Reasoning	-0.35 (1.48)	---
6. Verbal short-term memory	-0.17 (0.65)	---

* = Significant group difference (2 way independent t-test, $p < 0.05$)
[a] Age, Estimated IQ, BDI, BAI, Domain scores = Mean (Stdev.); Educational level, Marital Status, Menopausal status = n; Days since surgery: # of days between surgery and baseline scan; Domain scores = standardized to controls.
Abbreviations: IQ= Intelligence quotient, BDI = Beck Depression Inventory, BAI = Beck Anxiety Inventory

Table B **Significant t-test results comparing patients and controls for the a-x contrast**

Controls - Patients

Condition	Region	Coordinates	ROI P_{FWE}	K	T value	Z value
No covariate	L Cerebellar tonsil	-14 -48 -46 -20 -46 -46	0.030	15	3.74	3.47
Cortisol	L Cerebellar tonsil	-16 -50 -46 -16 -46 -48	0.025	20	4.14	3.56
Metacognition	None					
RXN time Press A	None					
Days surgery	L Cingulate Gyrus L Cingulate Gyrus L Middle frontal gyrus L Middle frontal gyrus	-14 -20 40 -6 -20 42 -38 28 48 -34 30 38	0.022 0.041	71 65	4.44 3.45 4.40 3.46	4.00* 3.22 3.98* 3.24
BDI	None					
BAI	None					
Errors of Commission	None					

[b] Table shows significant ROI investigation at threshold 0.001 uncorrected, P_{FWE} = 0.05 cluster-wise correction and k>10. N= 23 patients and 23 controls, except cortisol with 12 patients and 15 controls. (R = right, L = left); * indicates significance at Bonferroni adjustment for multiple comparisons.

Table C **Significant flexible factorial results comparing patients and controls for the x-rest and a-rest contrasts**

Controls - Patients

Condition	Region	Coordinates	$P_{FWE\ corr}$	T value	Z value
x-rest	R Cerebellar tonsil	32 -48 -40	0.000	10.09	7.22
	L Precentral gyrus	-26 -14 70	0.000	6.61	5.48
	R Superior temporal pole	52 12 -20	0.024	5.73	4.92
	R Supplementary motor area	14 -12 68	0.024	6.61	4.85
	R Fusiform	30 -80 -6	0.035	5.34	4.66
a-rest	R cerebellar culmen	38 -60 -36	0.000	9.39	6.92
	R cerebellar tonsil	20 -52 -40	0.000	6.74	5.56
	L cerebellar tonsil	-24 -50 -44 -26 -56 -38	0.000	7.36 6.82	5.91 5.60
	R Inferior occipital gyrus	40 -74 -10	0.007	6.11	5.17
	R Lingual gyrus	26 -88 -6	0.013	5.63	4.86
	R Fusiform	28 -82 -4	0.035	5.37	4.69

[c] Table shows significant activations at $P_{FWE} = 0.05$. N= 23 patients and 23 controls, except cortisol with 12 patients and 15 controls. (R = right, L = left)

Table D **Significant regression results for days since surgery and diurnal cortisol in patients only.**

Patients

Condition	Whole brain P_{FWE}	Coordinates	Region	ROI P_{FWE}	K	T value	Z value
Cortisol +	No	18 -8 -20	R Parahippocampus	0.045	18	7.82	4.34*
		22 -4 -36	R Parahippocampus	0.030	15	5.06	3.49
		-26 -4 -32	L Parahippocampus	0.010	62	6.55	3.99*
		-18 -10 -28	L Parahippocampus			4.39	3.21
		-26 2 -40	L Parahippocampus			5.28	3.57
		-16 2 -18	L Parahippocampus	0.048	13	5.82	3.76
		4 -40 -14	R Cerebellar culmen	0.008	16	5.59	3.68
		0 -34 -16	R Midbrain, brainstem	0.038	33	5.18	3.53
		2 -20 -26	R Brainstem, pons	0.041	27	4.74	3.36
		-4 -16 -26	L Brainstem, pons	0.053	10	4.58	3.29
Cortisol -	No	NA					
Days since surgery +	0.051	-32 22 48	L Middle frontal gyrus		184	6.11	4.58*
		-36 28 42	L Middle frontal gyrus			4.92	3.97*
		-32 14 46	L Middle frontal gyrus			4.48	3.71
		-34 28 38	L Middle frontal gyrus			4.40	3.66
		-32 14 46	L Middle frontal gyrus			3.69	3.20
	0.011	-12 -66 40	L Precuneus		273	5.64	4.35*
		-2 -6 40	L Precuneus			5.07	4.05*
		-12 -70 38	L Precuneus			5.05	4.04*
		-8 -72 40	L Precuneus			4.74	3.87
		-8 -70 36	L Precuneus			4.64	3.81
		-2 -78 46	L Precuneus			4.02	3.42
		-2 84 46	L Precuneus			3.99	3.40
		-10 -20 40	L Cingulate gyrus	0.004	137	5.20	4.12*
		-14 -26 44	L Cingulate gyrus			4.36	3.64
	0.057	-4 -18 38	L Cingulate gyrus			3.83	3.30
		-8 -14 50	L Paracentral lobule	0.034	13	3.90	3.34
	0.057	52 -48 14	R Superior temporal gyrus	0.022	62	5.14	4.09*
		62 -52 16	R Superior temporal gyrus			4.91	3.96
	0.074						
		62 -52 16	R Middle temporal gyrus	0.011	102	5.10	4.07*
		50 -48 12	R Middle temporal gyrus			5.00	4.01*
	0.074						
		-48 -50 6	L Middle temporal gyrus	0.035	60	4.89	3.95
		-50 -50 26	L Supramarginal gyrus	0.043	17	4.21	3.55
		34 26 -20	R Inferior orbitofrontal gyrus	0.073	11	4.08	3.46
		-12 20 70	L Supplementary motor area	0.063	17	3.97	3.39
		8 28 24	R Anterior cingulate	0.034	25	4.02	3.42
Days since surgery -	No	NA					

[d] Table shows whole brain investigation at P_{FWE} = 0.05 as well as ROI investigation at threshold 0.001 uncorrected, P_{FWE} = 0.05 cluster-wise correction and k>10.. N= 23 patients, 23 controls, except cortisol at 12 patients / 15 controls. (R = right, L = left; (+) = positive, (-)= negative),); * indicates significance at Bonferroni adjustment for multiple comparisons.

Figure 1- **Diurnal cortisol rhythms for breast cancer patients and matched controls**

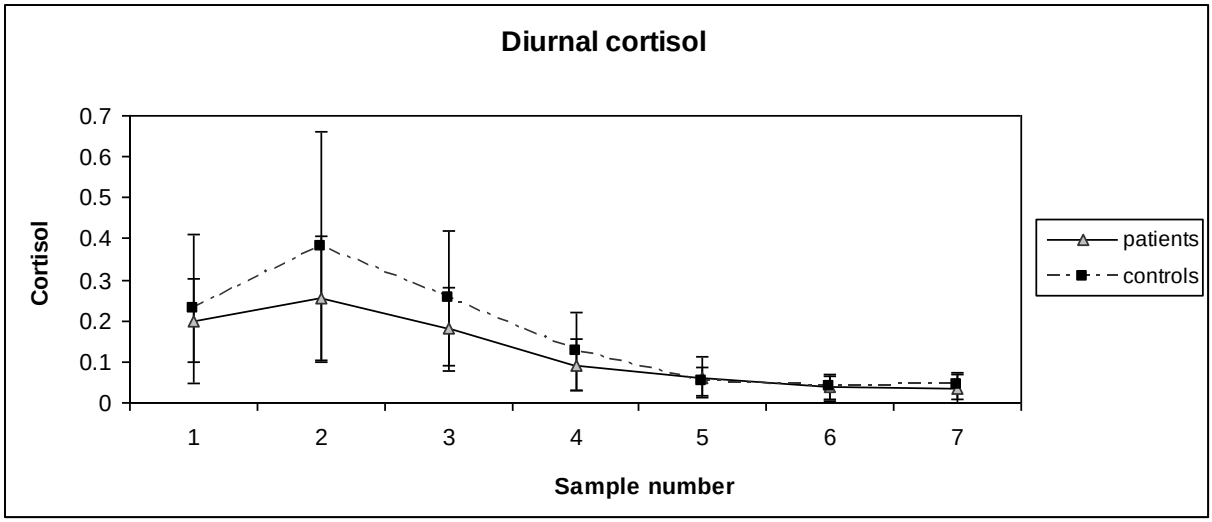
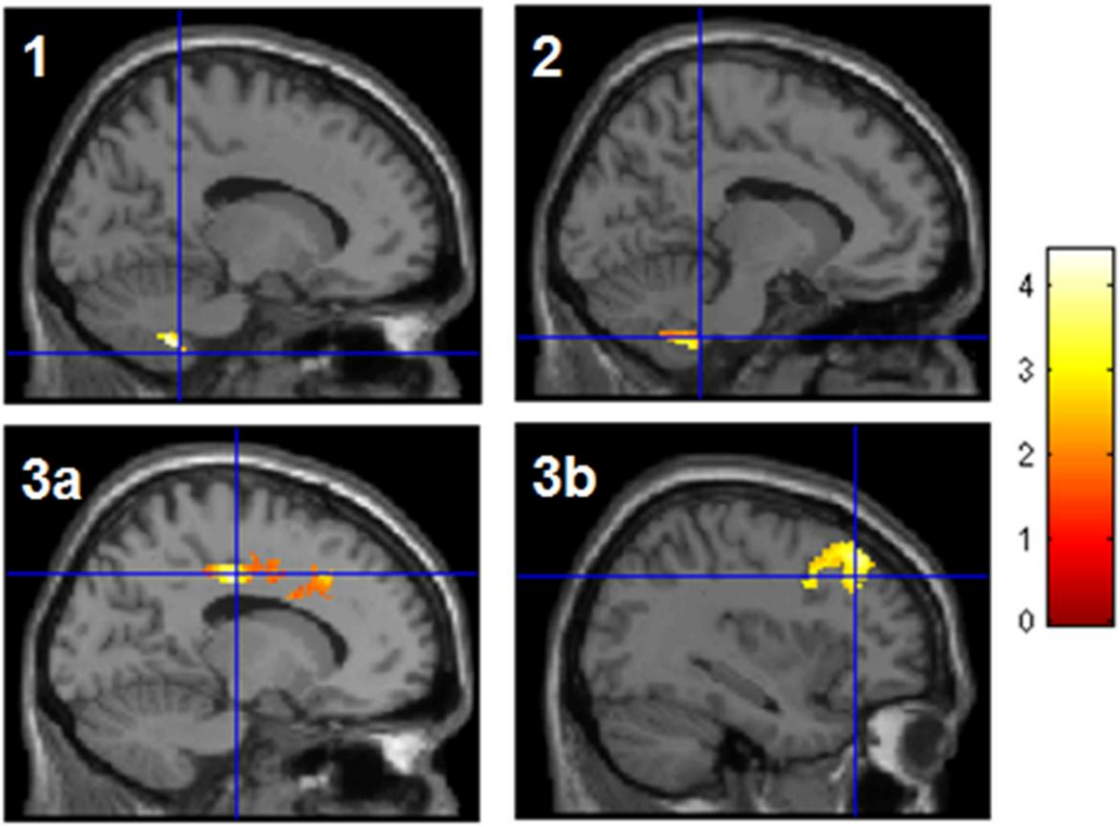
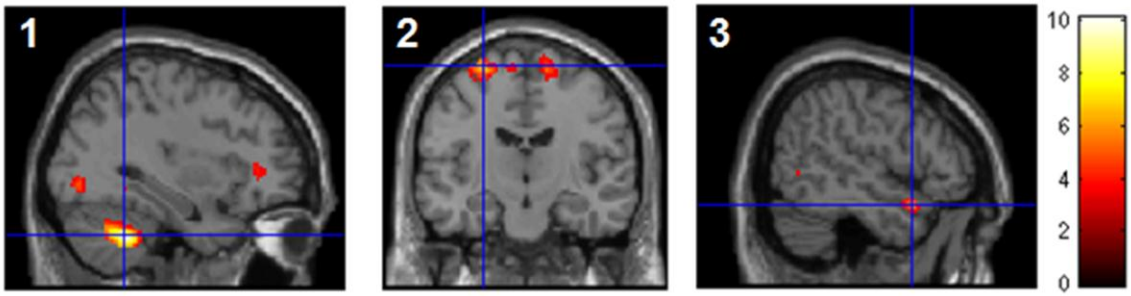


Figure 2- **Significant t-test results for controls – patients for the a-x contrast**



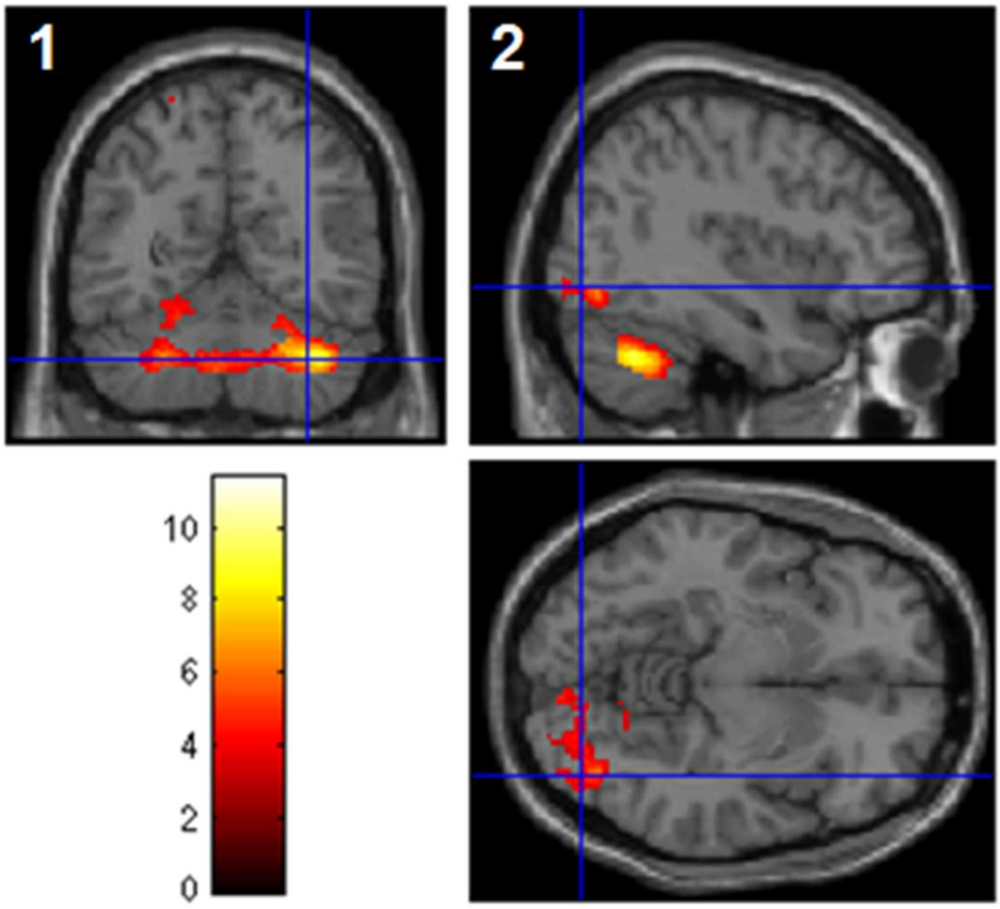
1. No covariate (L cerebellar tonsil)
2. Cortisol as covariate (L cerebellar tonsil)
3. Days since surgery as covariate (a. L cingulate gyrus, b. L middle frontal gyrus)

Figure 3- **Significant flexible factorial results for controls – patients for the x-rest contrast**



1. Right posterior cerebellar tonsil, right fusiform gyrus
2. Left precentral gyrus, right supplementary motor area
3. Right superior temporal pole

Figure 4- **Significant flexible factorial results for controls - patients for the a-rest contrast**



1. Bilateral cerebellar culmen and cerebellar tonsil
2. Right inferior occipital gyrus, right lingual gyrus, right fusiform

Chapter 6

General Discussion and Conclusions

The review paper in this thesis was the first to systematically assess neuroimaging studies of CRCIs (mostly in BC patients) as well as discuss possible confound variables that should be considered in future studies of the impact of BC on cognition, both pre- and post-chemotherapy. It also discusses the current limitations related to translation from research to clinical practice, and suggests a direction for future studies in this field. The three original papers presented in this thesis are the first imaging studies to systematically investigate pre-chemotherapy neuroanatomical and neurofunctional differences between BC patients and well matched controls. Additionally, it is strengthened by consideration of demographic, psychological and biological factors in the analyses. Results show pre-treatment structural and functional group differences, highlighting the importance of better characterizing BC patients and understanding potentially confounding variables before considering post-treatment effects.

This is particularly important in such a multifactorial population, where patients are not only subject to their cancer treatment, but also many other significant variables. The simple fact that not all patients experience CRCIs, and that these manifest at different intensities and lengths of time, indicates that there may be predisposing factors. It is possible that many of these factors could be present at baseline. Assessments of only post-chemotherapy may be attributing some of these baseline effects as side-effects of chemotherapy, and it is also possible that chemotherapy agents exacerbate subtle baseline group differences. This thesis focused on regions on interest (ROIs) that were consistently reported in post-chemotherapy studies. Although group differences were rarely revealed during stringent whole brain analyses, use of these ROI showed discrepancies between patients and controls. Requiring ROI analyses for significance may indicate that these group differences are subtle at baseline, however, the fact that these regions are significantly different in both pre- and post-chemotherapy studies signifies that baseline cognitive status may be carried over in the CRCI studies.

Uncovering which factors contribute to baseline group differences between BC patients and controls, or drive within-group heterogeneity indicates which variables could also be of importance for more systematic investigations post-chemotherapy. For example, when considering the number of days since surgery, there is a heterogeneity revealed in the BC patient population that was either not observed or manifests differently in controls. There was a positive relationship revealed between neural activity during response inhibition neural activity and the more days that had passed since surgery in the BC patients. Such a finding is interesting as it indicates a significant effect of surgery and this "effect" is manifesting before chemotherapy-treatment. This suggests that the length of time between the surgery date and commencement of chemotherapy treatment could contribute to the development and/or severity of CRCIs. If a patient has fewer days since surgery and chemotherapy, could the lingering effects of anesthesia and higher circulating cytokine levels increase their vulnerability to chemotherapy agents and their related side-effects? Future longitudinal post-chemotherapy assessments of this BC patient population will help shed light on this question. Such investigations will also take place along other confounding variables found to be significant in the other original structural and functional papers of this thesis.

Anecdotal evidence of chemofog has been partially substantiated by both neuroimaging and neuropsychological investigations. The BC population, however, is vulnerable to cognitive change even before chemotherapy and this has been the main finding of this thesis. Therefore, their executive functioning capacities necessary for factors such as decision making, an essential ability in this challenging time in their life, may already be compromised prior to chemotherapy. As discussed more in depth in the review paper, there are few investigations systematically investigating BC patients at baseline and there is a lack of test-retest studies post-chemotherapy which make it very difficult to translate research into clinical practice. Overall more longitudinal studies are required, with careful consideration of possible confounding variables at all stages of the study (prior to, during and post-chemotherapy). In particular, understanding these factors at baseline and their related side effects will lead to a clearer picture of impairments related to chemotherapy treatment itself. This may also help uncover possible

predisposing factors in the development of CRCIs. As the study of CRCIs becomes more prevalent, there needs to be a consensus concerning control group selection as well as functional tasks used. Consistency in the results would lead to clearer clinical applications for the thousands of Canadian women afflicted by breast cancer every year. The importance of this recognition for these women and their treatment cannot be overestimated. Patients with a new cancer diagnosis are faced with many challenges both biological (cytokine activity, anesthesia) and personal (stress, anxiety, depression) which deserve to be systematically studied.