CHEMOTHERAPY, ESTROGEN, AND COGNITION: NEUROIMAGING AND GENETIC VARIATION

Susan Kim Conroy

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Brenna C. McDonald, PsyD, MBA, Chair

Andrew J. Saykin, PsyD

Doctoral Committee

R. Andrew Chambers, MD

August 4, 2012

Kathy D. Miller, MD

Karmen K. Yoder, PhD

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Abstract

Susan Kim Conroy

CHEMOTHERAPY, ESTROGEN, AND COGNITION: NEUROIMAGING AND GENETIC VARIATION

The time course and biological mechanisms by which breast cancer (BC) and/or alterations in estrogen status lead to cognitive and brain changes remain unclear. The studies presented here use neuroimaging, cognitive testing, genetics, and biomarkers to investigate how post-chemotherapy interval (PCI), chemotherapy-induced amenorrhea (CIA), and genetic variation in the estrogen pathway affect the brain. Chapter 1 examines the association of post-chemotherapy interval (PCI) with gray matter density (GMD) and working memory-related brain activation in BC survivors (mean PCI 6.4, range 3-10 years). PCI was positively associated with GMD and activation in the right frontal lobe, and GMD in this region was correlated with global neuropsychological function. In regions where BC survivors showed decreased GMD compared to controls, this was inversely related to oxidative DNA damage and learning and memory scores. This is the first study to show neural effects of PCI and relate DNA damage to brain alterations in BC survivors. Chapter 2 demonstrates prospectively, in an independent cohort, decreased combined magnitudes of brain activation and deactivation from pre-to post-chemotherapy in patients undergoing CIA compared to both postmenopausal BC patients undergoing chemotherapy and healthy controls. CIA's change in activity magnitude was strongly correlated with change in processing speed, suggesting this activity increase reflects effective cognitive compensation. These results demonstrate that the pattern of change in brain activity from pre- to post-chemotherapy varies according to pre-treatment menopausal status. Chapter 3 presents the effects of variation in *ESR1*, the gene that codes for estrogen receptor-α, on brain structure in healthy older adults.

ESR1 variation was associated with hippocampus and amygdala volumes, particularly in females. Single nucleotide polymorphism (SNP) rs9340799 influenced cortical GMD and thickness differentially by gender. Apolipoprotein E (*APOE*)-ε4 carrier status modulated the effect of SNP rs2234693 on amygdala volumes in women. This study showed that genetic variation in estrogen relates to brain morphology in ways that differ by sex, brain region and *APOE*-ε4 carrier status. The three studies presented here explore the interplay of BC, estrogen, and cognition, showing that PCI, CIA, and *ESR1* genotype influence brain phenotypes. Cognitive correlates of neuroimaging findings indicate potential clinical significance of these results.

Brenna C. McDonald, PsyD, MBA, Chair

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Introduction

Breast cancer and cognition

Breast cancer (BC) will affect 1 in 8 American women, and the 5-year survival rate is 90% as of 2009 [1]. BC patients undergo many treatments, including surgery, cytotoxic chemotherapy, targeted therapies, radiation and hormonal treatments. As survivorship continues to increase, attention to the impact of BC and its treatments on patients' quality of life grows. Cognitive effects have been a recent area of focus. Post-chemotherapy neuropsychological deficits, observed in 13-70% of survivors [2], are subtle, typically occur in executive function, processing speed, and memory domains, and may persist for many years (for review and meta-analysis see [3-6]). Lower than expected cognitive performance has also been described in cancer patients before any treatment is initiated [7, 8], suggesting that host factors and the disease process itself also play a role. Subjective cognitive changes have also been reported in a range of studies, and interestingly are not highly correlated with objectively measured neuropsychological dysfunction (see [9] for review).

Several mechanisms have been proposed for cancer- and treatment-related cognitive dysfunction [10]. Chemotherapeutic agents increase oxidative stress, induce DNA damage, and may lead to decreased telomere length. Genetic and cancer- or treatment-induced alterations of blood-brain barrier integrity may influence the amount of direct neurotoxicity caused by chemotherapy. Genetic predisposition may leave some patients more vulnerable to (or less able to recover from) neural insult. The changes that occur in the body as a result of cancer and its treatments are complex. Systemic inflammation and changes in neuroendocrine activity and the interaction of these processes with the brain have been associated with neuropsychological and psychosocial dysfunction in cancer patients [11-13]. Increases in inflammatory processes increase overall oxidative stress. Dysregulation of cytokines and inflammatory processes may result from either cancer or treatment. For example, patients show increased serum levels of a protein associated with the inflammatory cytokine tumor

necrosis factor (TNF)-α one month post-chemotherapy [14]. Some cancer treatments directly or indirectly alter levels of hormones that are known to affect cognition, including estrogen and testosterone. All of these potential mechanisms, and possibly others, may combine in varying proportions to cause cognitive dysfunction in an individual cancer patient.

Study of animal models has yielded valuable insights (see [15] for review). Rats with induced mammary tumors show increased hippocampal expression of the cytokines interleukin (IL)-1β, IL-6, IL-10, and tumor necrosis factor (TNF)-α in the absence of any cancer treatment [16]. Oligodendrocytes and their precursors appear to be particularly vulnerable, and delayed myelin damage has been observed following several types of chemotherapy [17, 18]. Methotrexate treatment decreases hippocampal neurogenesis and alters hippocampal vasculature in rats without outright cell death 12 weeks post-treatment regardless of tumor presence or absence [19-21]. Cyclophosphamide appears to impair long-term potentiation (LTP) during treatment, with some post-treatment recovery [22]. Cerebellar cells show alterations in cytoskeletal and calcium regulating proteins and cortical cells show dendritic retraction in response to cytosine arabinoside (AraC) treatment [23, 24].

Neuroimaging of cancer- and treatment-related changes

Neuroimaging is a valuable tool in the assessment of neural effects of cancer and its treatments. Neuroimaging of chemotherapy-related cognitive dysfunction has several potential uses, including providing biomarkers for future identification of patients at risk and stimulating hypotheses for basic science investigations which could ultimately lead to neurorehabilitative or neurotherapeutic agent development. Further, imaging may detect alterations that are not evident in neuropsychological testing, as the subtlety of cancer and treatment effects on cognition often lead to neuropsychological test scores in the normal range. The most important research tool for structural imaging to date has been anatomic magnetic resonance imaging (MRI). For gray matter (GM), a technique called

voxel-based morphometry (VBM) is often employed in analyzing MR images. VBM is a non-biased, fully automated technique that can assess density within a tissue compartment (GM, white matter (WM), or cerebrospinal fluid (CSF)) throughout the brain. VBM and other voxel-by-voxel comparisons with other imaging modalities typically utilize statistical parametric mapping (SPM), a method which calculates statistical differences between groups and/or over time at each voxel in the brain, with both peak magnitude and spatial extent (number of voxels) considered in determining statistical significance of a particular cluster [25]. White matter analyses have utilized diffusion tensor imaging (DTI), to assess both magnitude and directionality of water diffusion (see [26] for review). As water flows preferentially along the direction of myelinated WM tracts (as opposed to orthogonally), DTI allows assessment of WM tract integrity, which can be disrupted in many neurological disorders.

Functional imaging has employed both positron emission tomography (PET) and functional MRI (fMRI). PET imaging relies on the injection of trace amounts of a radioactive compound to assess a particular aspect of brain function at rest or during activity. For example, the most commonly used tracer, (^{18}F)-fluorodeoxyglucose [(^{18}F)-FDG], a glucose analog, is used to assess regional brain metabolic activity, while (^{15}O)-H_2O is used to measure changes in blood flow. Blood oxygenation level dependent (BOLD) (fMRI) is a noninvasive (i.e., contrast-free) method used to detect regional brain activation in response to presentation of a stimulus or performance of a cognitive task. During a particular cognitive function, a characteristic anatomical pattern of activation is observed as task-related areas of the brain become more active. At the same time, regions of the default mode network (DMN) are deactivated in a complementary process as neural resources are reallocated [27]. Magnitudes of both activation and deactivation increase parametrically with task difficulty [28, 29]. Activated brain regions show increased blood flow, peaking about five seconds after stimulus presentation. This increase in blood flow is called the hemodynamic response, and locally increases the amount of deoxyhemoglobin present; deoxyhemoglobin

is paramagnetic and perturbs the magnetic field, thus serving as an endogenous contrast agent for detecting neuronal activity [30]. Pathology can manifest as compensatory hyperactivation or failure to activate sufficiently, as illustrated by the early, higher functioning or later, lower functioning stages of mild cognitive impairment (MCI), respectively [31, 32]. Thus, association of fMRI alterations with neurocognitive performance and self-reported cognition is important in interpretation of results.

Structural studies have revealed brain changes in GM in BC patients. Two recent prospective studies in independent cohorts from our laboratory [33, 34] have shown relatively decreased GM density, particularly in the frontal lobes, in BC patients undergoing chemotherapy from pre-treatment to one month post-chemotherapy completion compared to both BC patients not receiving chemotherapy and healthy controls. Interestingly, one report showed a relationship between change in self-reported executive function and change in GM density [34]. No clinically meaningful differences among these three groups were evident at baseline in either study. One study followed patients a year later and found that GM density partially recovered but did not return baseline levels [33]. Retrospective studies have shown longer-term changes in GM. The first retrospective VBM study of chemotherapy effects showed diffusely distributed decreases in cortical GM in cancer survivors at least five years post-treatment compared to healthy controls [35]. Another study demonstrated decreased frontal and medial temporal GM in chemotherapy-treated BC patients an average of 4 months post-treatment compared to non-chemotherapy-treated patients, and GM in these regions was correlated with attention and memory measures. In a somewhat overlapping cohort, no differences were present an average of 2.6 years post-chemotherapy [36]. Nine years post-treatment, BC survivors exposed to chemotherapy showed decreased GM in posterior parietal and cerebellar regions compared to non-chemotherapy-treated BC survivors [37], while survivors an average of 21 years post-treatment showed a decrease in total GM volume but no focal decreases evident on VBM compared to cancer-free controls

[38]. In summary, chemotherapy appears to negatively affect GM, most prominently in the frontal lobes, with effects that are most pronounced in the months following chemotherapy. These effects have been associated with both subjective and objective cognitive function. Partial GM recovery appears to occur in the following years to decades. Pre-treatment GM differences between BC patients and controls have not been apparent.

Several studies have also explored treatment-related changes in WM. A single prospective DTI study has shown decreased white matter integrity from pre- to 3-5 months post-chemotherapy in BC patients. Non-chemotherapy-treated patients and healthy controls did not show changes over time, and there were no differences between the three groups at baseline. Interestingly, frontal WM change was associated with change in verbal memory score, and parietal WM change was associated with change in working memory score [39]. Retrospectively, a VBM study of WM in cancer survivors showed distributed decreases in subcortical WM density compared to healthy controls [35]. Patients with memory complaints an average of two years post-chemotherapy showed decreased DTI WM integrity in the genu but not splenium of the corpus callosum compared to healthy controls in a region-of-interest analysis, and decreased genu integrity was associated with slower processing speed [40]. Another retrospective VBM study showed lower WM in distributed regions four months post-chemotherapy compared to non-chemotherapy-treated BC patients; precuneus WM was associated with attention scores. In the same study in a partially overlapping cohort VBM found no WM differences in chemotherapy-treated patients 2.6 years post-chemotherapy. Nine years post-chemotherapy DTI has revealed lower WM integrity in widely distributed tracts compared to non-chemotherapy-treated BC patients [37]. Finally, a study of BC patients 3-5 months post-chemotherapy showed lower WM integrity in frontal and temporal WM tracts compared to non-chemotherapy-treated BC patients and healthy controls. These alterations were correlated with attention, processing speed, and self-reported cognitive function [41]. In summary, chemotherapy appears to

negatively affect WM density and tract integrity, an effect that persists for years and perhaps decades following treatment. WM alterations are associated with poorer subjective and objective cognitive function. There is no evidence for pre-treatment alterations in WM in BC patients. WM alteration is consistent with the processing speed alterations often observed post-chemotherapy.

PET studies have shown physiological changes related to cancer and treatment. In an FDG-PET study, cancer patients in 4 categories (pre-chemotherapy, post-chemotherapy, recurrent, and terminal) showed resting hypometabolism in distributed brain regions compared to healthy controls. Interestingly, the pre-chemotherapy group showed the greatest degree of hypometabolism [42]. Another FDG-PET study did not reveal any differences 5-10 years post-chemotherapy compared with non-chemotherapy-treated BC patients, although resting metabolism in the left inferior frontal cortex of BC patients was positively correlated with performance in visuospatial memory [43]. In the same study, ^{15}O-H_2O imaging showed relatively greater blood flow during a verbal memory task in chemotherapy-treated patients, with peak blood flow change in the inferior frontal gyrus.

Both cancer- and treatment-related effects have been detected with fMRI. A pretreatment study showed greater inferior frontal and parietal and lower anterior cingulate activity during a working memory task in BC patients compared to healthy controls. Task performance was lower in the patients [44]. In the first prospective longitudinal fMRI study in BC, our laboratory showed pre-treatment frontal hyperactivation and parietal hypoactivation during a working memory task in BC patients compared to healthy controls [45]. BC patients treated both with and without chemotherapy showed relative decreases in frontal regions from pre- to one month post-chemotherapy (or an equivalent interval in non-chemotherapy-treated patients). One year later, both groups showed increased frontal activity, partially returning to their baseline hyperactive state. In the chemotherapy group, the left frontal region of maximal change overlapped with a region demonstrating

decreased GM density in the same cohort [33], suggesting a structure-function relationship, and performance on the in-scanner working memory task was decreased one month post-chemotherapy, suggesting that frontal hyperactivation was compensatory.

Retrospective fMRI studies have also proved informative. An initial report of monozygotic twins discordant for BC and chemotherapy showed greater activation throughout task-activated regions in the affected twin 22 months post-chemotherapy. Task performance was similar in both twins, suggesting compensatory hyperactivation in the BC twin [46]. A study of BC survivors nine years post-chemotherapy showed decreased frontal and parietal activation during an executive function task compared to non-chemotherapy-treated BC survivors, and frontal activation in the chemotherapy group was significantly positively correlated with in-scanner task performance [47]. In the same study, fMRI during an episodic memory task showed lower parahippocampal gyrus and parietal activation in the chemotherapy group. Subsequent performance on a recognition task of items encoded during the in-scanner task was correlated with parahippocampal gyrus activation. A subsequent study on the same cohort showed striking anatomic overlap of both GM and WM chemotherapy-associated alterations with chemotherapy-affected regions on fMRI [37]. In chemotherapy-treated BC patients a mean of three years post-treatment, lower frontal activation during a memory encoding task was observed compared to healthy controls. However, during memory retrieval, BC patients showed diffusely greater activation. Performance on the in-scanner memory task was similar between groups [48]. Finally, five years post-treatment, BC patients treated with or without chemotherapy showed lower activation in several prefrontal regions during an executive function task compared to healthy controls. In another prefrontal region activation was lower in chemotherapy-treated BC patients compared to non-chemotherapy-treated patients and healthy controls, suggesting that a greater degree of hypoactivation relative to healthy controls occurred in chemotherapy-treated BC patients compared to non-chemotherapy-treated patients. Behavioral

measures were correlated with imaging results in this study: in chemotherapy-treated patients, lower prefrontal activation was associated with greater subjective executive function complaints, and in healthy controls greater activation was associated with lower executive function complaints and faster executive function task performance [49].

Taken together, fMRI studies show a pattern of pre-treatment and early post-treatment compensatory frontal activation. Frontal changes are consistent with working memory and executive function changes observed in BC. Many years post-chemotherapy, frontal hypoactivation is present compared to either non-chemotherapy-treated or cancer-free controls, which might reflect late sequelae of chemotherapy. In many of these studies, neurocognitive performance was correlated with alterations in brain function. Several studies show evidence of anatomically overlapping structural and functional effects.

In summary, neuroimaging studies of BC patients show post-chemotherapy structural changes and both pre- and post-chemotherapy functional changes. The frontal lobes appear to be the most broadly affected. Many alterations seen on imaging have shown neurocognitive correlates, suggesting functional relevance. These studies occurred at a range of post-treatment intervals, from months to decades. The trajectory of such changes over time, i.e., the effect of post-chemotherapy interval, has not been formally studied. Also unclear is the neural impact of systemic estrogen changes in breast cancer patients resulting from chemotherapy-induced amenorrhea (CIA) or hormonal treatments. As it is critical to brain function, estrogen likely plays an important role in cancer- and treatment-related cognitive dysfunction in BC.

Estrogen and the brain

The cognitive and neural effects of estrogen across the lifespan are an active area of research, as estrogen is critically involved in memory processing. Steroid hormone levels decrease with age in both men and women [50, 51]. The

menopausal transition is characterized by a loss of ovarian-produced estrogen in females around the age of 50, and cognition during and after this period has been a particular focus of study. Up to 60% of women report cognitive problems associated with menopause [52], and longitudinal studies have shown dysfunction peaking during the perimenopausal period [53, 54]. Age-related decreases in circulating estrogen, particularly in association with the menopausal transition, have adverse cognitive effects [55, 56]. Estrogen loss reduces neuroplasticity and longer duration of estrogen deprivation (i.e., more time after menopause) is associated with worse outcomes [57]. The “healthy cell bias theory of estrogen action” posits that estrogen replacement would be effective only before estrogen loss has injured the cell beyond repair via dysregulation of mitochondrial and bioenergetic processes [58]. This is consistent with studies showing that the cognitive benefits of hormonal replacement therapy (HRT) appear to depend on timing and formulation of treatment [59, 60]. Also, abrupt estrogen loss in pre-menopausal women, either by oophorectomy or treatment with gonadotropin releasing hormone (GnRH) agonists, has been linked to cognitive dysfunction [61].

The pleiotropic actions of estrogen in the brain suggest a host of potential biological mechanisms for estrogen depletion-related cognitive dysfunction. While the majority of circulating estrogen in premenopausal females is produced by the ovaries, neural estrogen is produced by the enzyme aromatase in neurons, astrocytes, and cerebrovascular endothelial cells in both genders [62]. Both estrogen receptor-α (ER-α) and ER-β are distributed throughout the brain. Estrogen is neurotrophic and neuroprotective in vitro and in vivo [62]. Estrogen is anti-inflammatory in the brain via reduction of microglial activation, and is neuroprotective in ischemia and oxidative stress [63]. Neuroplastic processes, including synaptogenesis and neurogenesis, are regulated by estrogen [57]. The genes of several neural growth factors, including *BDNF* and *VEGF*, have estrogen response elements, thus altering their expression in response to an estrogen stimulus [64]. Estrogen also interacts with many neurotransmitter

systems, including acetylcholine, serotonin, glutamate, and dopamine [65], and regulates vasodilation of cerebral blood vessels [66]. Estrogen downregulates several molecular pathways associated with Alzheimer's disease (AD) pathology. Estrogen is associated with: decreased amyloidogenic processing of amyloid precursor protein (APP) [67, 68], which may lead to decreased amyloid plaque deposition; inhibition of the enzyme glycogen synthase kinase-3β, which is critical in creating the hyperphosphorylated tau that makes up neurofibrillary tangles; and increased expression of choline acetyltransferase (ChAT), an enzyme known to be deficient in AD, leading to impaired cholinergic neurotransmission [69]. The involvement of estrogen in this variety of neural processes underlies its functional importance in higher-level processes such as cognition.

In light of the estrogen pathway's involvement with many crucial neural processes, it is not surprising that genetic variation leading to alterations in estrogen synthesis, response, or degradation has been implicated in cognition and cognitive disorders. *ESR1*, the gene coding for estrogen receptor (ER)-α, has been particularly well studied. Many studies have shown association of *ESR1* polymorphisms with decreased cognitive function or risk of dementia (for systematic review, see [70]). Polymorphisms in *ESR1* have also been shown to interact with the apolipoprotein E (*APOE*) ε4 allele, the genetic marker conferring greatest AD risk [71-73]. Risk of dementia has also been associated with polymorphisms in *ESR2* [74, 75], which codes for ER-β, and *CYP19A1* [76-78] which codes for aromatase, the enzyme that synthesizes estrogen from testosterone (see [70, 79] for review).

Neuroimaging has provided insight about brain changes that underlie the relationship between estrogen and cognition [80] via comparison of high-estrogen and low-estrogen states. Structural MRI studies have found diminished gray matter in postmenopausal women not using HRT vs. those currently using HRT [81-85], although some have found no such relationship [86]. fMRI studies

have demonstrated a pattern of lower task-related activation in postmenopausal women not receiving HRT relative to those who are [87-91], in younger women after gonadotropin releasing hormone agonist (GnRHa)-induced ablation of ovarian estrogen production [92-94], and during the low as compared to high estrogen phase of the menstrual cycle [95, 96]. These functional changes occur most commonly in prefrontal, temporoparietal, precentral, and paracentral regions. Several studies using single photon emission computerized tomography (SPECT) or (^{15}O)-H_2O PET have shown lower cerebral blood flow in postmenopausal women not receiving HRT as compared to postmenopausal women receiving HRT [97-101] and premenopausal women [100]. Overall, decreased levels of estrogen have been associated with lower gray matter density, task-related brain activation, and cerebral blood flow.

Hormonal changes in breast cancer

Breast cancer treatment can influence systemic estrogen in several ways. First, 80% of pre- or peri-menopausal breast cancer (BC) patients undergoing current widely-used chemotherapy regimens (cyclophosphamide and doxorubicin, with or without a taxane) experience chemotherapy-induced amenorrhea (CIA) in the months immediately following chemotherapy [102-105]. CIA results from disruption of normal ovarian follicular maturation, leading to markedly decreased systemic estrogen levels [106], and is associated with increased survival due to the loss of estrogen's trophic effects on estrogen receptor-positive tumors [102, 107]. CIA negatively impacts quality of life, as patients undergoing CIA appear to experience more severe symptoms than women undergoing natural menopause [108]. Prospective studies have shown decline or failure to improve with practice in multiple cognitive domains in patients undergoing CIA compared to patients undergoing chemotherapy but not amenorrhea [109, 110], although other studies found no such effect [111-113]. Timing of measurements appears to play a role. As time passes after chemotherapy, 20-60% of women pre- or peri-menopausal before treatment experience continued amenorrhea [105, 114, 115]. Many factors influence resumption of menses, including tamoxifen therapy [115].

For the approximately 75% of breast cancer patients who have estrogen receptor-positive tumors, a several-year course of an aromatase inhibitor (AI) or selective estrogen response modulator (SERM) such as tamoxifen is recommended to prevent recurrence. AIs block the production of estrogen throughout the body and thus decrease circulating estrogen levels, while SERMs act as estrogen agonists or antagonists, depending on the tissue, and do not affect circulating estrogen levels [116].

In BC patients cognitive dysfunction has been associated with both AIs and SERMs [85, 117-121], although other studies have failed to demonstrate such a relationship [111, 122-126]. AIs and SERMs have different neural effects as observed in animal models. Decreased aromatase activity, either through AI administration or knock-out of the aromatase gene, has been associated with decreased neurogenesis, increased damage from excitotoxicity, and increased ischemic damage [65]. SERMs, on the other hand, appear to have a variety of neurotrophic effects, although estrogen agonistic or antagonistic activity varies among brain regions and between SERMs. Some SERMs have antioxidant and anti-inflammatory properties in the brain [127].

A few cross-sectional, retrospective imaging studies have explored the neural effects of tamoxifen. Decreased resting metabolism in the basal ganglia has been demonstrated in breast cancer patients with a history of tamoxifen and chemotherapy as compared to patients receiving chemotherapy but not tamoxifen, patients receiving neither chemotherapy nor tamoxifen, and healthy controls [43]. Breast cancer survivors receiving tamoxifen also showed resting hypometabolism in frontal and postcentral regions compared to women without cancer regardless of HRT status [85]. An MR spectroscopy study found lower overall myoinositol levels in women receiving tamoxifen or HRT as compared to postmenopausal women receiving neither. Since increased myoinositol is associated with injury-related increases in glial activity, this study concluded that tamoxifen and HRT have neuroprotective effects [128]. Overall, the little existing

literature suggests resting hypometabolism in BC patients using tamoxifen. However, no neuroimaging studies of AI treatment or CIA exist, nor are there any prospective neuroimaging studies of treatment-related hormonal changes in BC.

Current Studies and Significance

The precise time course and biological mechanisms by which BC and/or alterations in estrogen status lead to cognitive and brain changes remain unclear. The following three chapters use advanced neuroimaging, cognitive testing, genetics, and biomarker analysis to investigate the ways in which post-chemotherapy interval, chemotherapy-induced amenorrhea, and genetic variation in the estrogen pathway affect the brain.

Despite a number of retrospective studies and several emerging prospective studies of the effects of chemotherapy on brain structure and function, the time course of neural changes that occur during survivorship is unclear. Chapter 1 of this document examines the association of post-chemotherapy interval (PCI) with gray matter density and working memory-related brain activation in BC survivors with PCIs ranging from 3-10 years (mean 6.4). The relationships among PCI, neuroimaging, cognitive changes and biological markers could help elucidate important mechanisms. We hypothesized that in BCS compared to healthy controls, there would be increased cognitive dysfunction, changes on neuroimaging, and oxidative and direct DNA damage and that these would be associated with shorter PCI. Notably, this study is the first to address the relationship between peripherally measured oxidative stress and brain structure in BC, possibly providing a mechanistic clue to cognitive dysfunction in BC patients.

Chemotherapy-induced amenorrhea (CIA) accompanied by loss of systemic estrogen often occurs in pre- and peri-menopausal BC patients, and while cancer/chemotherapy and abrupt estrogen loss have separately been shown to affect cognition and brain function, studies of the cognitive effects of CIA are equivocal, and its effects on brain function are unknown. Chapter 2 prospectively investigates the effects of CIA by comparing patients undergoing CIA to BC patients already postmenopausal at chemotherapy initiation using fMRI prior to and one month after chemotherapy completion. We hypothesized that the CIA

profile of activation and deactivation during a working memory task would be distinct from that of post-menopausal patients undergoing chemotherapy or healthy controls. We assessed the relationship between changes in brain activity and cognition in order to determine the functional significance of observed neural effects.

Chapter 3 addresses the role of estrogen in the brain more generally. Cognitive and neurodegenerative phenotypes have been associated with genetic variation in estrogen pathway genes, particularly *ESR1*, the gene that codes for estrogen receptor-α. We used structural imaging to determine the effect of *ESR1* polymorphisms on brain structure in healthy older male and female adults from the Alzheimer's Disease Neuroimaging Initiative (ADNI). We hypothesized that *ESR1* genotype could explain variance in hippocampal and amygdalar volumes and whole-brain gray matter density, and that these effects would vary by gender. In light of epidemiological and molecular evidence, we also hypothesized that *APOE*-ε4 genotype would interact with *ESR1* in influencing brain phenotypes.

The findings from the above studies may have future implications for informed treatment decisions by BC patients and their physicians. By studying the impact of estrogen loss during CIA, we hope to add to the literature on brain changes in BC that has previously focused almost completely on chemotherapy effects. Our findings will also contribute to a more general understanding of neural estrogen's role in cognition and cognitive dysfunction. We believe that these studies will be a step toward future development of neuroprotective or rehabilitative interventions, either pharmacological or behavioral, for detrimental BC- and estrogen-related neural effects.

Chapter 1: Alterations in brain structure and function in breast cancer survivors: effect of post-chemotherapy interval and relation to oxidative DNA damage

Neuroimaging studies have begun to uncover the neural substrates of cancer and treatment-related cognitive dysfunction, but the time course of these changes in the years following chemotherapy is unclear. In this chapter, we analyzed multimodality 3T MRI scans to examine the structural and functional effects of chemotherapy and post-chemotherapy interval (PCI) in a cohort of breast cancer survivors (BCS; n=24; PCI mean 6, range 3-10 y) relative to age- and education-matched healthy controls (HC; n=23). Assessments included voxel-based morphometry (VBM) for gray matter density (GMD) and functional MRI (fMRI) for activation profile during a 3-back working memory task. The relationships between brain regions associated with PCI and neuropsychological performance, self-reported cognition, and peripheral oxidative and direct DNA damage were assessed in secondary analyses. Cognitive complaints and oxidative DNA damage were increased, and memory performance decreased, in BCS compared to HC. Imaging results indicated lower GMD and fMRI activation in several regions in the BCS group. PCI was associated with GMD and activation in the right anterior frontal region (Brodmann Areas 9 and 10) independent of participant age. GMD in this region was also correlated with global neuropsychological function. In regions where BCS showed decreased GMD this was inversely related to oxidative DNA damage and learning and memory neuropsychological domain scores. This is the first study to show structural and functional effects of PCI and to relate DNA damage to brain alterations in BCS. The relationship between neuroimaging and cognitive function indicates the potential clinical relevance of these findings. The relationship with peripheral oxidative DNA damage suggests a potential mechanism of chemotherapy-related neural changes warranting further investigation.

Introduction

Increasing evidence shows cognitive changes related to breast cancer and its treatments, as demonstrated by lower than expected performance on neuropsychological tests. Changes occur particularly in executive function (including working memory) and processing speed domains (for meta-analysis and review see [3-6]). Self-report measures show increased perceived cognitive difficulties in cancer patients (see [9] for review).

Imaging studies have recently begun to reveal the neural substrates of such changes. Retrospective MRI studies have shown post-chemotherapy structural alterations in both gray [36-38] and white matter [40, 41] in cohorts of breast cancer survivors (BCS) whose average post-chemotherapy intervals (PCIs) ranged from four months to 21 years. Retrospective functional MRI (fMRI) studies have shown hypoactivation during cognitive tasks, at an average of 4.7 years [49] or 10 years [47] post-chemotherapy, compared to BCS who never received chemotherapy. De Ruiter et al. demonstrated links between structural and functional results in in a single cohort approximately 10 years post-chemotherapy (compared to non-chemotherapy-treated BCS), with gray and white matter changes overlapping with regions of fMRI hypoactivation [37]. Recently, prospective structural, functional and diffusion tensor MRI studies [33, 39, 45] have shown changes after treatment, particularly in the frontal cortex. The recent prospective data is particularly important given reports of cognitive [7] and functional [44, 45, 129] changes prior to treatment.

Many biological mechanisms for cancer- and treatment-associated cognitive dysfunction and brain changes have been proposed; prominent among them are oxidative stress, DNA damage, and compromised DNA repair, all of which may be shared risk factors for development of cancer and sensitivity to cancer- and treatment-related neurological side effects [10]. Many chemotherapeutic agents are known to produce DNA damage and/or free radicals, which cause oxidative damage. Increased oxidative and endogenous DNA damage in white blood cells

have been demonstrated in women with breast cancer pre-treatment, and these changes are exacerbated after chemotherapy [130].

Despite recent progress, the time course of changes during survivorship is poorly understood. The current study examines the association of PCI with gray matter density (GMD) and working memory-related functional MRI (fMRI) brain activation in BCS. The relationships among PCI, neuroimaging, cognitive changes and biological markers could help elucidate important mechanisms of chemotherapy-related neural changes. We hypothesized that in BCS compared to healthy controls there would be increased cognitive dysfunction, changes on neuroimaging, and oxidative and direct DNA damage, and that these would be associated with shorter PCI.

Methods

Participants

BCS with a history of non-metastatic disease and chemotherapy treatment and age- and education-matched healthy controls (HC) were recruited as part of a larger study of the cognitive sequelae of cancer and its treatments [131, 132], so the sample had prior assessment with a subset of neuropsychological measures 2-3 years earlier. In the present study, 27 BCS and 25 HC underwent structural and functional MRI exams as well as a battery of neuropsychological tests and self-report measures. Blood was obtained for biomarker analysis, including comet assays to assess DNA damage (see below). Four participants were excluded from all analyses: one HC for current use of antipsychotic medication, two BCS for history of stroke, and one BCS for extremely poor performance (>4 SD below the control mean) on neuropsychological tests that was thought to be due to a comorbid condition.

Image Acquisition, Processing, and Analysis

All images were acquired on the same Siemens MAGNETOM Tim Trio 3T scanner using a 12-channel head coil as previously described [34, 45]. Structural scans

were a sagittal T1-weighted MP-RAGE (magnetization prepared rapid gradient echo) sequence with the parameters: 160 contiguous 1.2mm slices, TR: 2300ms, TE: 2.91ms, TI: 900ms, flip angle: 9, NEX: 1, BW/Pixel: 240, FOV: 256mm, matrix 256x256, in-plane resolution: 1.0×1.0 mm. Blood oxygenation level-dependent (BOLD) fMRI images were acquired axially with a T2*-weighted single shot echo-planar imaging (EPI) pulse sequence with the parameters: TR: 2250ms, TE: 29ms, flip angle: 79, FOV: 220mm, matrix: 88×88, slice thickness: 3.5mm, NEX: 1, yielding 39 contiguous axial slices and a voxel dimension of 2.5x2.5x3.5mm.

As in our prior fMRI studies of breast cancer patients [45, 46] a block design verbal “N-back” task was used. During scanning, participants saw a series of consonant letters (except L, W, and Y), presented one every three seconds. Task conditions were 0-, 1-, 2-, and 3-back. Each condition was presented in 27-second epochs preceded by three seconds of instruction (e.g., “the match is one back”). For each letter participants responded via button press to indicate whether the current letter was a match (i.e., was the same as the designated target or the letter presented 1, 2, or 3 back in the sequence, depending on the condition) or a non-match. The four experimental conditions were each presented three times in pseudorandom order for a total of 12 task blocks (total duration = 6:54). Participants practiced a version of the task prior to scanning to ensure comprehension of the task. Presentation software (Neurobehavioral Systems, Inc., Albany, CA) was used to program the task and record response accuracy and reaction times.

Image processing for VBM used in-house MATLAB (Version 7.9 (R2009b), Mathworks, Inc., Natick, MA) scripts to implement optimized VBM methods [133-135] using Statistical Parametric Mapping software (SPM; Version 8, Wellcome Department of Imaging Neuroscience, London, UK), similar to our prior studies [33, 45, 136-138]. Briefly, after reconstruction, scans were registered to the Montreal Neurological Institute T1-weighted template and segmented into gray matter, white matter, and cerebrospinal fluid compartments using the MNI T1-

weighted template and corresponding tissue probability maps. Gray matter maps were then spatially normalized to MNI space, resampled to 1mm isotropic voxels, and smoothed using an isotropic Gaussian spatial filter (FHWM = 10mm). The smoothed, normalized gray matter maps were used for second-level multi-subject voxelwise analyses. The SPM8 prior probability gray matter template was used to restrict statistical comparisons to the gray matter compartment.

fMRI image processing also utilized SPM8. Spatial realignment using a six parameter model was performed on raw scan data to remove minor motion-related signal change. Realignment parameters were entered as covariates at the subject level, and all volumes were normalized into standardized atlas space, resampled to 2 mm^3 isotropic voxels, and smoothed to a FWHM of 8mm. Contrast images comparing pairs of working memory load conditions (e.g., 3-back > 0-back) were created for each participant. These contrast images were then used in second-level multi-subject voxelwise analyses. Mixed model analyses accounted for both random effects (scan) and fixed effects (task condition).

Voxelwise random effects analyses for both VBM and fMRI were conducted using linear regression (for PCI) or t-tests (between-group analysis) as implemented in SPM8 to construct maps of voxels in which local GMD or activation differed as a function of PCI or between BCS and HC groups. Age was included as a covariate in all group analyses. Statistical significance was set at an uncorrected voxel-level p_{crit} of 0.001 and a minimum cluster size (k) of 10 voxels. In fMRI comparisons, a mask of the main effect of the 3-back > 0-back comparison (p_{crit} = 0.05) in all participants was applied in order to search in only regions of task-related activation.

In order to correlate imaging data with behavioral and comet assay data, MarsBaR v0.42 (http://marsbar.sourceforge.net/) was used to extract mean GMD or activation values from each statistically significant cluster. To reduce

dimensionality of imaging data from each analysis, these mean cluster values were averaged to create an overall mean value of significant clusters for each analysis.

Neurocognitive Testing and Self-Report Measures

Raw neurocognitive test scores were normalized using the mean and standard deviation of the HC group scores. Domain scores were created for each participant by averaging the z-scores of the included tests. Domain scores were then adjusted for age. The learning domain consisted of the Rey Auditory-Verbal Learning Test (AVLT) total learning score [139, 140], story recall (immediate) [141], and the sum of recalled items for initial learning trials on the Brown Learning Test (BLT) [142]. The memory domain comprised Rey AVLT delayed recall, story recall (10 minute delay) [141], and BLT long delay score. The attention domain included Wechsler Adult Intelligence Scale (WAIS-III) Digit Span forward total score [143] and the Rao Paced Auditory Serial Addition Test (PASAT) 2 and 3 minute trial total scores [144]. The language domain included the Wide Range Achievement Test (WRAT-4) Word Reading test [145] and the Wechsler Abbreviated Scale of Intelligence (WASI) Vocabulary test [146]. The visuospatial domain was the WASI Block Design raw score. The executive domain consisted of the Digit Span backward total score, the Controlled Oral Word Association (COWA) Test total score [139, 140], Delis-Kaplan Executive Function System (D-KEFS) Color-Word Interference Test inhibition and inhibition/switching trials times [147], D-KEFS Sorting Test number of correct sorts, and D-KEFS Trail Making Test number-letter switching trial time. The psychomotor domain consisted of D-KEFS Color-Word Interference Test color naming trial time, D-KEFS Trail Making Test number sequencing trial time, Symbol Digit Modalities Test oral total score [148], and Grooved Pegboard total time [149].

Self-reported cognition was assessed using the Multiple Ability Self-Report Questionnaire (MASQ) [150] and the Functional Assessment of Cancer Therapy-

Cognitive Function (FACT-COG) [151]. It should be noted that, unlike other self-report data presented, higher scores on the FACT-COG are indicative of *decreased* cognitive complaints. The Center for Epidemiologic Studies-Depression Scale (CES-D) [152] and the State-Trait Anxiety Inventory-State Scale (STAI-S) [153] were used to assess depression and anxiety symptoms.

Comet Assay

The comet assay for assessment of direct and oxidative DNA damage was performed as described previously with modifications [154-156]. For white blood cells (WBC), immediately after the blood samples were taken, 2 µl whole blood was mixed with 70 µl 1% low-melting agarose and applied onto Trevigen CometSlides (Trevigen, Gaithersburg, MD). After the gel solidified, cells were lysed (100mM Na_2EDTA, pH 10, 2.5M NaCl, 10mM Trizma base, 1% sodium lauryl sarcosinate, 1% Triton-X 100, 10% DMSO, and 1mM deferoxamine; 1 h), placed in alkali buffer (0.3M NaOH and 1mM Na_2EDTA, pH > 13; for 40 min at 4°C), and then electrophoresed in alkali buffer (25 V, 300 mA, 30 min). Following electrophoresis, slides were neutralized (0.4M Tris, pH 7.5, at 4°C for 5 min), rinsed in distilled water (at 4°C for 15 min), and allowed to dry at room temperature in the dark. After slides were stained with ethidium bromide (25 µg; 20 µg/ml) and covered with a cover slip, a total of 100 nuclei/treatment selected at random per slide were evaluated on a Nikon fluorescence microscope using Komet 4.0 imaging Software (Kinetic Imaging Ltd., Liverpool, UK). DNA damage was expressed as comet (Olive) tail moment ([tail mean - head mean] x tail%DNA/100) [154, 157]. For the assessment of oxidative DNA damage, a modified alkaline comet assay was performed that included enzymatic digestion with formamidopyrimidine DNA glycosylase (fpg) prior to electrophoresis. Briefly, after the lysis of cells, slides were washed (40mM 4-[2- hydroxyethyl]piperazine-1-ethanesulfonic acid, 100mM KCl, 0.5mM Na_2EDTA, and 0.2% bovine serum albumin; pH 8.0, 5 min, 3x) and then incubated with fpg solution or buffer (100 µl for 40 min at 37°C). Slides then underwent alkaline unwinding and the remainder of the procedure as described above.

Statistical Analyses

Linear regression within BCS only was used to assess the effect of PCI on self-reported cognition, neuropsychological test scores, and DNA damage. T-tests were used to assess significance of between-group effects with these same variables. To examine the possible pattern of relationships between imaging results and other key variables, we tested the association between mean GMD and activation values with the above measures separately in each group using regression analyses. For these secondary analyses, a liberal significance threshold of $p \leq 0.05$ was employed due to the relatively small sample size and likely non-independence among cognitive variables.

Results

Demographic information, self-report, comet assay, neuropsychological domain, and cancer- and treatment-related information are shown in Table 1-1. Groups did not differ in age, education, handedness, *APOE* status, estimated IQ, STAI-S, or CES-D scores. Patients were an average of 6.4 years post-chemotherapy (range: 3.2-10.2). PCI was not significantly correlated with age ($r = -0.086$, $p = 0.688$).

Table 1-1 Demographic, self-reported cognition, DNA damage, neuropsychological domain, and breast cancer treatment information. Values shown are mean ± SD; asterisks indicate between group $p \leq 0.05$.

	Healthy Control	Breast Cancer Survivors
N	23	24
Age (years)	61.2±9.9	57.8±9.6
Education (years)	16.0±2.3	15.7±2.1
Handedness (R:L)	19:4	22:2
APOE e4 allele positive	16	16
Full Scale IQ Estimate (Barona Index [158])	113.4±3.9	113.3±4.3
CES-D raw score	8.7±6.9	7.5±5.8
STAI-S raw score	31.9±9.1	30.2±7.9
3-back score (corrected for guessing)	54.1±21.9	53.7±21.2
3-back reaction time (seconds)	0.772±0.186	0.825±0.200
FACT-COG total raw score*	135.3±13.0	116.2±28.8
MASQ total raw score*	79.9±17.7	97.1±20.3
Direct DNA damage (alkaline comet assay tail moment)	1.0±0.3	0.8±0.3
Oxidative DNA damage (fpg comet assay tail moment)*	1.0±0.6	1.5±1.2
Age-adjusted neuropsychological domain z-scores:		
Learning	0.2±0.7	-0.2±0.7
Memory*	0.03±0.5	-0.3±0.6
Attention	0.1±0.7	0.4±0.6
Language	-0.03±0.9	0.3±0.8
Visuospatial	0.1±0.9	-0.5±1.0
Executive	0.04±0.6	-0.04±0.7
Psychomotor	0.04±0.4	-0.1±0.4
Average	0.1±0.4	-0.1±0.5

Patient Information		
Age at diagnosis		51.2±10.1
Received radiation		19
Received tamoxifen		13
Received aromatase inhibitor		17
Average post-chemotherapy to MRI interval (years)		6.4±2.1 (range 3.2-10.2)
Stage I number of patients (percent)		7 (29)
Stage IIa		8 (33)
Stage IIb		6 (25)
Stage IIIa		2 (8)
Stage IIIb		1 (4)
Chemotherapy regimens:		
Doxorubicin, cyclophosphamide (AC)		7
Doxorubicin, cyclophosphamide, taxane (AC-T)		5
Doxorubicin, cyclophosphamide, 5-fluorouracil (CAF)		2
Doxorubicin, taxane (A-T)		3
Cyclophosphamide, methotrexate, 5-fluorouracil (CMF)		2
CMF and CAF		1
Taxane only		1
AC-T and capecitabine		2
Taxane and capecitabine		1

Post-chemotherapy interval

Imaging

In voxelwise regressions with PCI (BCS group only), structural and functional effects converged anatomically in the right anterior frontal lobe (Figure 1-1): GMD was positively correlated with PCI in the right superior and middle frontal gyri (Brodmann Areas (BA) 9, 10) and activation was negatively correlated with PCI in the right middle frontal gyrus (BA 10). Greater GMD was associated with longer PCI in 10 clusters within the bilateral frontal and parietal lobes and basal ganglia, as well as the right temporal lobe (Table 1-2). No regions were evident in which lower GMD was associated with longer PCI or in which activation was positively correlated with PCI.

Figure 1-1 Functional and structural overlap in the right anterior middle frontal gyrus, in which gray matter density (red) was positively correlated with post-chemotherapy interval and working memory-related activation (blue) was negatively correlated with post-chemotherapy interval (voxelwise p uncorrected = 0.001, cluster size=10 for both modalities).

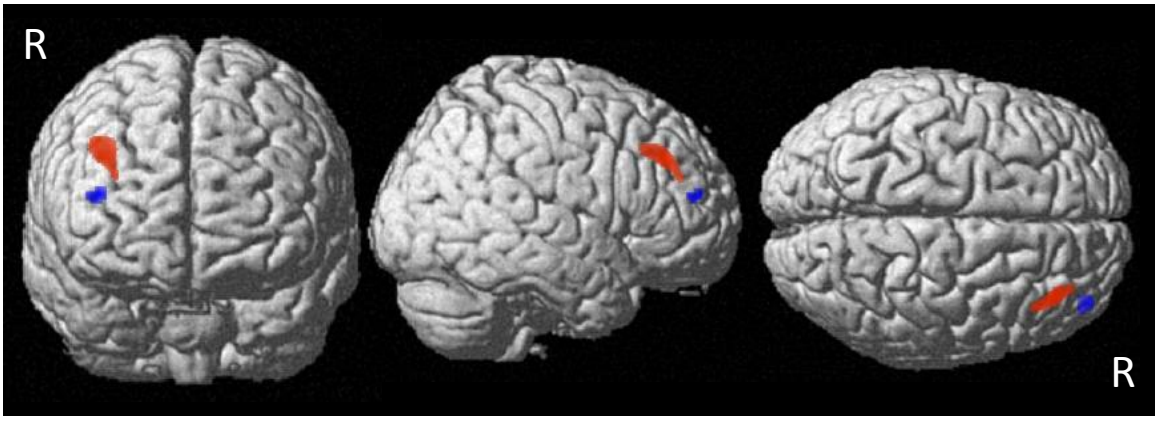


Table 1-2 Statistically significant clusters from imaging analyses (voxel-level p uncorrected = 0.001, cluster size = 10 voxels for all analyses).

Peak MNI coordinates (x y z)	Cluster extent (k)	Cluster-level $p_{corrected}$ (FWE)	Cluster-level $p_{uncorrected}$	Z	Region description of local maxima within cluster	Brodmann Area (BA)
VBM: Positive Correlation with time since chemotherapy						
9 9 42	79	0.996	0.465	4.15	Right cingulate gyrus	32
64 8 8	160	0.970	0.294	4.10	Right precentral gyrus	44
37 37 33	608	0.453	0.051	4.09	Right superior and middle frontal gyri	9, 10
20 21 5	73	0.997	0.484	3.63	Right caudate	N/A
-8 16 47	48	0.999	0.577	3.50	Left cingulate gyrus	32
-21 11 -23	105	0.991	0.396	3.44	Left inferior frontal gyrus	47
-21 20 2	11	>0.999	0.814	3.44	Left putamen	N/A
15 -80 33	26	>0.999	0.694	3.42	Right precuneus	7
-9 83 14	37	0.999	0.630	3.38	Left cuneus	18
64 -20 -2	40	0.998	0.615	3.35	Right superior temporal gyrus	21
3-back > 0-back: Negative correlation with time since chemotherapy						
40 52 16	16	0.922	0.095	3.84	Right middle frontal gyrus	10
VBM: Healthy Control > Breast Cancer Survivor						
-43 -67 8	143	0.971	0.411	3.67	Left middle temporal gyrus	37
16 -26 -4	188	0.948	0.344	3.60	Right midbrain	N/A
-14 -23 6	408	0.762	0.167	3.58	Left thalamus	N/A
2 -23 -17	184	0.950	0.349	3.53	Right midbrain	N/A
38 -66 -39	461	0.709	0.143	3.48	Right cerebellum	N/A
49 -21 23	59	0.995	0.611	3.37	Right insula	13
3-back > 0-back: Healthy Control > Breast Cancer Survivor						
10 -68 42	23	0.808	0.080	3.56	Right precuneus	7
-28 -66 22	15	0.954	0.150	3.47	Left middle temporal gyrus	39

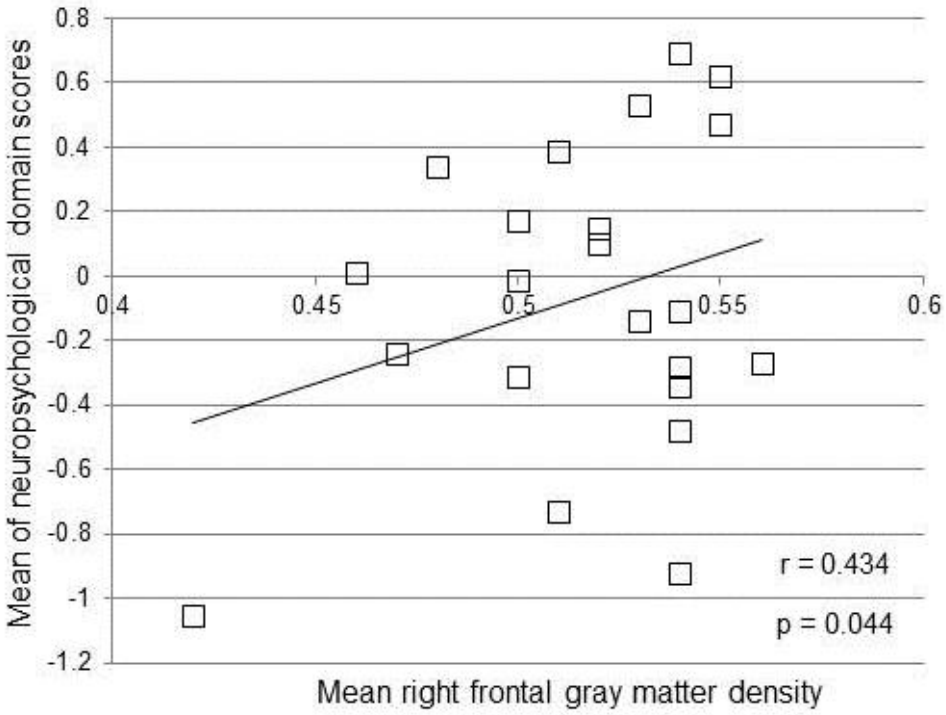
3-back task performance and reaction times, cognitive testing, self-reports, and comet assay

PCI was not significantly correlated with 3-back task performance or reaction time, any neuropsychological domain or self-report measure, or DNA damage.

Relationship of imaging to other measures

In secondary analyses, the mean GMD value of the right frontal cluster depicted in Figure 1-1 (red) was positively correlated with the overall neuropsychological performance (mean domain score, r = 0.434, p = 0.044) (Figure 1-2). The mean activation of the 3-back cluster depicted in Figure 1-1 (blue) was not associated with any other measures at $p \leq 0.05$. The average of all GMD clusters derived from a positive correlation with PCI was not associated with any other measures at $p \leq 0.05$.

Figure 1-2 In breast cancer survivors (BCS), the average of neuropsychological domain z-scores vs. mean gray matter density in right middle and superior frontal gyri (Brodmann Areas 9, 10).



Between-group analyses

Imaging

Imaging analyses revealed differences between the BCS and HC groups. BCS showed decreased GMD relative to HC in multiple regions including the left temporal lobe, right midbrain, left thalamus, right cerebellum, and right insula (Table 1-2). BCS did not show any regions of increased GMD relative to HC. Functional imaging revealed relatively decreased working memory-related brain activation in BCS compared to HC in the right precuneus and left middle temporal gyrus. No areas were associated with relatively greater activation in BCS.

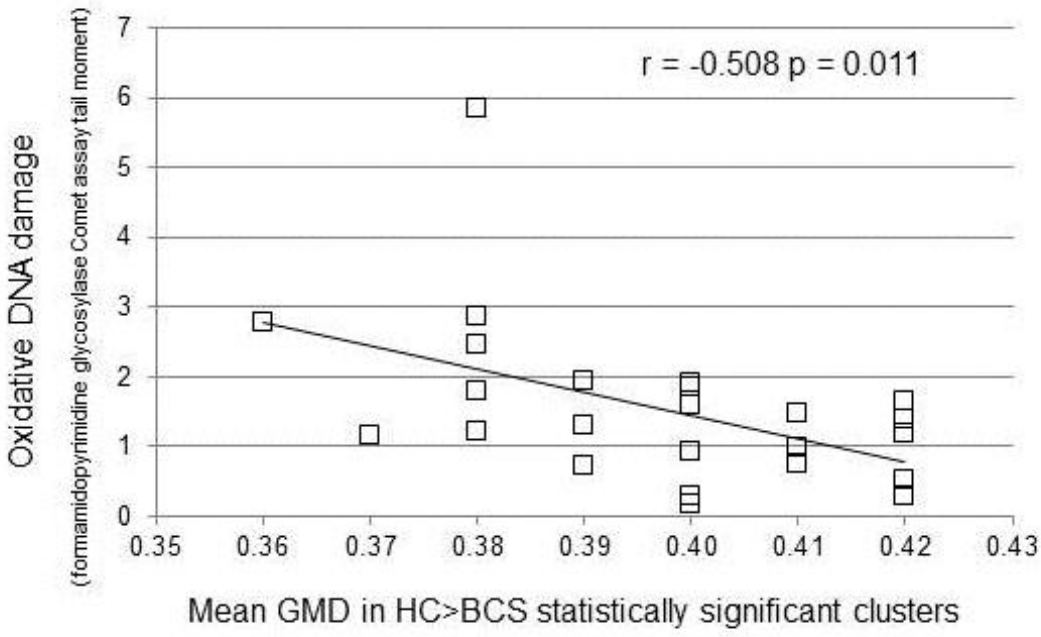
3-back task performance and reaction times, cognitive testing, self-reports, and comet assay

Performance accuracy and reaction times for the 3-back task did not differ between groups. BCS scored lower than HC on the memory domain on neuropsychological testing ($F(1, 45) = 4.20$, $p = 0.046$). The BCS and HC groups also differed on the FACT-COG ($F(1, 45) = 8.45$, $p = 0.006$) and MASQ ($F(1, 45) = 9.61$, $p = 0.003$) self-report measures, with BCS reporting more cognitive complaints on both measures. Oxidative DNA damage was increased in BCS ($F(1, 45) = 4.00$, $p = 0.052$) (Table 1-1).

Relationship of imaging to other measures

Within the BCS group, overall neuropsychological function and all but two domains were uncorrelated with GMD in the mean of the regions showing between-group differences. The learning and memory domain scores, however, were negatively correlated with GMD ($r = -0.639$, $p = 0.001$ and $r = -0.647$, $p = 0.001$, respectively). Increased oxidative damage was associated with lower GMD in BCS ($r = -0.508$, $p = 0.011$, Figure 1-3). Activation did not correlate with any other measure. Within the HC group, no significant correlations were found.

Figure 1-3 Oxidative DNA damage in breast cancer survivors (BCS) as measured by comet assay of peripheral lymphocytes vs. mean gray matter density (GMD) of clusters in which BCS showed relatively decreased GMD compared to healthy controls (HC).



Discussion

This multimodal MRI study of long-term BCS found PCI associated with both structural and functional changes on brain MRI. Regional changes on imaging were in turn related to neuropsychological performance. Furthermore, a plasma biomarker showed increased oxidative DNA damage in BCS compared to controls which was also inversely associated with structural changes.

GMD was positively correlated with PCI in distributed brain regions with the largest clusters in the frontal lobes. This suggests that frontal gray matter recovers over time after an initial chemotherapy insult, as indicated by two prior prospective studies [33, 34]. Working memory-related brain activation was inversely associated with PCI in a region proximal to the largest structural finding. The convergence of structural and functional changes in the frontal region is consistent with prior reports of changes in executive cognitive functions after chemotherapy [3-6, 159] as well as retrospective [47, 49] and prospective [45] functional neuroimaging studies. Results of previous functional imaging studies in breast cancer patients have been interpreted as showing compensatory hyperactivation in similar frontal regions [44-46, 129]. The present results suggest that frontal hyperactivation diminishes over time, becoming less necessary as structural recovery proceeds. Alternatively, decreased activation with a longer PCI may reflect a failure of compensatory brain activation over time. Although neuropsychological performance could potentially help address this issue, the current study cannot differentiate between these mechanisms in the absence of substantial cognitive deficits after 6 years. Alterations in compensatory processes over time have been observed in other cognitive disorders. For example, in early, higher functioning stages of mild cognitive impairment (MCI), patients show hippocampal and prefrontal memory-related hyperactivation, and activation in these regions decreases as cognitive processes decline [31, 32].

Comparisons between BCS and controls indicated decreases in GMD and activation in left middle temporal gyrus. Taken together, the structural and

functional findings indicate an incomplete recovery after an average of 6.4 years post-treatment. This is generally consistent with previous GMD findings at a range of PCIs [36-38] as well as fMRI reports of hypoactivation 5-10 years after chemotherapy in BCS [47, 49] . Of note, there is also evidence for increased blood flow on ^{15}O-H_2O during a memory task an average of 5-10 years after chemotherapy [43].

Relationships among imaging and cognitive variables are a particularly interesting aspect of this study that increases the potential clinical significance of our findings. Greater GMD in a right frontal cluster derived from a voxelwise regression with PCI was associated with better global neuropsychological function. GMD clusters derived from between-group imaging analyses showed an unexpected negative correlation between GMD and learning and memory scores.

DNA damage in cancer patients is attributable to multiple factors, including cancer-related inflammation and oxidative stress, deficits in DNA repair mechanisms, and treatment-related insult [10]. Chemotherapeutic regimens can damage DNA in several ways [10, 160]. Direct DNA damage may occur as a result of the mechanism of action of a drug; for example, doxorubicin intercalates with DNA, causing strand breaks. Introduction of chemotherapeutic agent into a cell can also lead to generation of free radicals, resulting in oxidative damage. Substantial increases in both oxidative and direct DNA damage measured via peripheral lymphocyte comet assay have been reported from before to shortly after chemotherapy (1-2 weeks), and both oxidative and direct DNA damage were increased in BC patients relative to HC before treatment initiation [130]. In the present study, the increased oxidative but not direct DNA damage in BCS an average of 6 years post-chemotherapy relative to HC may suggest that oxidative damage is longer-lasting in this population. However, lack of correlation between oxidative DNA damage and PCI suggests a variable time course. Increased oxidative DNA damage in BCS was associated with decreased GMD in clusters

derived from between-group VBM analyses. This is an interesting finding with implications for the mechanism of chemotherapy-related brain changes, and warrants further investigation. Notably, increases in oxidative and direct DNA damage measured via peripheral lymphocyte comet assay have been also been reported in Parkinson's disease [161] as well as both mild cognitive impairment and Alzheimer's disease [162], suggesting that a link between oxidative DNA damage and neurodegeneration may be a common phenomenon.

Limitations of the present study include a retrospective, cross-sectional design that does not permit firm conclusions with regard to change over time. Our patient cohort had a history of several different chemotherapy regimens, although many agents were common across patients. Sample size does not allow for differentiation of effects of individual regimens. Further, our BCS experienced a range of time courses and types of endocrine treatments, which have been shown to affect cognition differentially from chemotherapy [118, 119, 163], and a majority of this cohort was using these therapies at the time of the study. Cohort size does not permit conclusions about these effects of these therapies.

To our knowledge this is the first imaging study of the effects of PCI on brain structure and function. We employed multi-modal MRI in concert with neuropsychological testing, cognitive self-report and biomarkers to examine the relationship among these variables, and found points of anatomic convergence. The potential clinical significance of imaging findings was underscored by association with cognitive measures. The link between neuroimaging variables and peripheral oxidative DNA damage may provide a direction for further mechanistic experiments. Prospective multi-modal investigations of treatment and survivorship will continue to be important as will recruitment of larger cohorts to enhance statistical power and generalizability. Multi-center collaborations may be required to achieve sufficient recruitment, which has proven difficult in this population. Although challenges remain to fully understand the time course of cognitive and biological changes after chemotherapy, the present study

demonstrates the important role that PCI may play in neural outcomes after chemotherapy.

Acknowledgements

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Chapter 2: Chemotherapy-induced amenorrhea: a prospective study of brain activation changes and neurocognitive correlates

Chemotherapy-induced amenorrhea (CIA) often occurs in pre- and peri-menopausal BC patients, and while cancer/chemotherapy and abrupt estrogen loss have separately been shown to affect cognition and brain function, studies of the cognitive effects of CIA are equivocal, and its effects on brain function are unknown. Functional MRI (fMRI) during a working memory task was used to prospectively assess the pattern of brain activation and deactivation prior to and one month after chemotherapy in BC patients who experienced CIA (n=9), post-menopausal BC patients undergoing chemotherapy (n=9), and pre- and post-menopausal healthy controls (n=6 each). Neurocognitive testing was also performed at both time points. Repeated measures general linear models were used to assess statistical significance, and age was a covariate in all analyses. We observed a group-by-time interaction in the combined magnitudes of brain activation and deactivation ($p = 0.006$): the CIA group increased in magnitude from baseline to post-treatment while other groups maintained similar levels over time. Further, the change in brain activity magnitude in CIA was strongly correlated with change in processing speed neurocognitive testing score ($r=0.837$ $p=0.005$), suggesting this increase in brain activity reflects effective cognitive compensation. Our results demonstrate prospectively that the pattern of change in brain activity from pre- to post-chemotherapy varies according to pre-treatment menopausal status. Cognitive correlates add to the potential clinical significance of these findings. These findings have implications for risk appraisal and development of prevention or treatment strategies for cognitive changes in CIA.

Introduction

Cancer and its treatments have been linked to cognitive dysfunction, particularly in the executive function, working memory, and processing speed domains [6]. Approximately 80% of pre- or peri-menopausal breast cancer (BC) patients undergoing current widely used chemotherapy (CTx) regimens (cyclophosphamide and doxorubicin, with or without a taxane) experience chemotherapy-induced amenorrhea (CIA) in the months immediately following CTx [102-105]. CIA results from disruption of normal ovarian follicular maturation, leading to markedly decreased systemic estrogen levels [106], and is associated with increased survival [102, 107]. As abrupt estrogen loss in pre-menopausal women has been linked to cognitive dysfunction [61], it is plausible that CIA may lead to increased detrimental effects of CTx compared to women who undergo CTx but not CIA (usually BC patients post-menopausal before CTx). Indeed, prospective studies have shown decline or failure to improve with practice in multiple cognitive domains in patients undergoing CIA compared to patients undergoing CTx but not amenorrhea [109, 110], although other studies found no such effect [111-113]. Timing of measurements appears to play a role.

Prospective functional neuroimaging has the power to observe, in the face of a neural insult such as CTx or estrogen loss, how the brain might compensate (in the context of maintained cognitive performance), or fail to adapt (in the context of decreased performance). We recently showed pre-treatment frontal hyperactivation in BC during a working memory task, with a decrease in activation in this region one month post-CTx accompanied by decreased working memory performance [45]. Performance and activation returned to higher levels one year later. The neural effects of abrupt estrogen loss in pre-menopausal women have been studied prospectively with gonadotropin hormone releasing hormone (GnRH) agonists. These studies generally show that estrogen ablation is associated with reversibly decreased task-related activation [92-94, 164]. However, the neural effects of CIA remain unclear.

The aim of this study was to prospectively measure global changes in working memory-related activation and deactivation, before cancer treatment and one month post-CTx completion. During a cognitive task, brain activation increases in "task-positive network" regions, while task-induced deactivation occurs in the anatomical regions of the "default mode network" (DMN) in a reallocation of neural resources [27]. Both activation and deactivation are important in cognition, and both are affected by normal aging as well as pathological conditions. Drawing on participants in our previous prospective fMRI study of BC patients [45], we examined working memory-related activation and deactivation in BC patients who underwent CIA (i.e., pre- or perimenopausal patients) and patients who were post-menopausal at CTx initiation. Pre- and post-menopausal healthy control (HC) groups were imaged at yoked intervals. We hypothesized that the CIA profile of activation and deactivation during a working memory task would be distinct from that of HC or post-menopausal CTx, and these changes over time would be correlated with changes seen in neurocognitive performance.

Methods

Participants

Appropriate cases were selected from our previous fMRI study [45] to fit the following categories: post-menopausal BC patients undergoing CTx (n=9), pre- or peri-menopausal BC patients undergoing CTx (n=9), pre- and post-menopausal HC (n=6 each). Scans and neuropsychological measures were collected prior to any cancer treatment (baseline; BL) and one month post-CTx completion (M1) or yoked intervals for HC.

Patients had stages I, II, or IIIA BC treated with standard-dose AC or AC-T CTx regimens (Table 2-1). Exclusion criteria included prior cancer or cancer treatment and medical, neurological, and psychiatric risk factors known to affect brain structure or function, as detailed previously [33]. Depressive symptoms and anxiety were assessed with the Center for Epidemiologic Studies-Depression

Scale (CES-D) [152] and the State-Trait Anxiety Inventory-State subscale (STAI-S) [153].

Table 2-1 Demographics and cancer treatment information

		Chemotherapy-induced amenorrhea (n=9)	Healthy control pre-menopausal (n=6)	Chemotherapy post-menopausal (n=9)	Healthy control post-menopausal (n=6)
Age (years)**		45.3±5.8 (range 35-55)	44.8±4.0 (range 37-48)	58.7±4.4 (range 53-69)	55.2±4.0 (range 50-59)
Education (years)		14.8±1.8	15.8±1.3	15.2±3.3	15.8±2.0
Handedness (R:L,A)		8:1	4:2	8:1	6:0
Full Scale IQ Estimate [158]		110.5±6.8	113.8±3.0	112.7±6.6	114.8±2.9
CES-D	BL	8.2±9.0	7.6±6.1	8.0±7.4	2.3±1.6
	M1	11.0±11.5	7.2±5.8	9.8±9.2	4.2±4.8
STAI-S	BL	34.1±14.0	28.6±8.6	29.0±9.1	25.2±7.5
	M1	35.7±18.2	28.0±5.3	28.4±8.8	27.5±11.9
Psychiatric Medications	BL	2 (1 buspirone and desipramine, 1 zolpidem)	0	2 (1 venlafaxine and buspirone, 1 fluoxetine)	0
	M1	3 (1 buspirone and desipramine, 1 zolpidem, 1 sertraline)	0	2 (1 venlafaxine and buspirone, 1 fluoxetine)	0
BL-M1 interval (days)		153±45	167±36	181±78	206±51
Cancer stage		2 stage I 5 stage II 2 stage IIIa		2 stage I 7 stage II	
Chemotherapy regimen		7 AC-T, 1 AC-T plus trastuzumab, 1 AC		7 AC-T, 2 AC	
Hormonal medication	BL	1 estradiol (for perimenopausal symptoms)	0	0	4 hormone replacement therapy
	M1	2 tamoxifen	0	1 tamoxifen, 1 aromatase inhibitor	2 hormone replacement therapy

Menstrual Status				
Periods regular throughout study	0	6	0	0
Post-menopausal (>6 months) throughout study	0	0	9	6
Periods irregular at BL, amenorrhea ≥6 months at M1	3	0	0	0
Periods regular at BL, amenorrhea ≥6 months at M1	4	0	0	0

** $p<0.001$ overall ANOVA; in post-hoc Tukey tests, both pre-/peri-menopaual groups less than both postmenopausal groups; both pre-/peri- and post-menopausal groups not different than each other
CES-D: Center for Epidemiologic Studies-Depression Scale [152]
STAI-S: State-Trait Anxiety Inventory-State subscale [153]
BL: baseline
M1: one month post-chemotherapy completion or yoked interval
AC: Doxorubicin and cyclophosphamide
AC-T: Doxorubicin, cyclophosphamide, and a taxane

Written informed consent was obtained according to the Declaration of Helsinki under a protocol approved by the Dartmouth College Committee for the Protection of Human Subjects.

MRI Scans

Scans were acquired on a 1.5T GE Signa LX scanner with echospeed gradients and standard head coil. A gradient-echo, echo-planar sequence provided whole brain coverage for fMRI: TR=2500ms, TE=40ms, FOV=24cm, NEX=1, 29 interleaved 5mm thick contiguous sagittal slices, yielding a 64x64 matrix with $3.75mm^2$ in-plane resolution. Structural scans were acquired to rule out incidental pathology and for the previously reported gray matter density analysis [33].
An auditory-verbal "N-back" task was used as in our previous study [45]. During scanning participants heard a string of consonant letters (except L, W, and Y) presented one every three seconds. Task conditions were 0-, 1-, 2-, and 3-back, in a blocked design. For each consonant participants used a button press device to signify whether the current letter was a match (i.e., was the same as the designated target or the letter presented 1, 2, or 3 back in the sequence) or a non-match. Each condition was presented in 27-second epochs preceded by three seconds of instruction (e.g., "the match is one back"). The four experimental conditions were each presented three times in pseudo-random order for a total of 12 task blocks. Participants rehearsed a practice version of the task prior to scanning to ensure comprehension of task demands. Stimuli were presented through an MRI compatible headphone system, and programmed in Presentation software (Neurobehavioral Systems, Inc., Albany, CA), which recorded response accuracy and reaction times.

Using raw scan data, spatial realignment using a six parameter model was performed in SPM5. Realignment parameters were entered as covariates at the subject level, and volumes were normalized into MNI space, resampled to $2mm^3$ voxels, and smoothed to a FWHM of 8mm. Statistical parametric mapping on a voxel-by-voxel basis was conducted using a general linear model (GLM)

approach. Contrast images comparing pairs of working memory load conditions (e.g., 3-back > 0-back) were created for each subject.

To derive numerical values for activation and deactivation during the 3-back task, all 3-back > 0-back contrast images were entered into a one-sample t-test in SPM5. Maps were created for activation (contrast vector 1; i.e., 3-back > 0-back) and deactivation (contrast vector -1; i.e., 0-back > 3-back) at $p_{crit} = 0.05$, cluster minimum size (k) = 10 voxels. MarsBar v. 0.42 [165] was used to extract mean values for each participant's activation and deactivation at both time points.

Neurocognitive Testing Domains

Raw neurocognitive test scores were normalized using the mean and standard deviation of the larger HC group from which HC subjects for the current study were drawn (demographic data regarding the larger cohort can be found in [33]). Domain scores were created for each subject by averaging the z-scores of the included tests, and were then adjusted for age using the entire sample of the current study. The verbal domain was calculated using the Wide Range Achievement Test (WRAT-3) reading score [166], the Wechsler Abbreviated Scale of Intelligence (WASI) Vocabulary score [146], and the Delis-Kaplan Executive Function System (D-KEFS) Verbal Fluency Test phonemic and semantic trial scores [147]. The verbal memory domain consisted of the California Verbal Learning Test total and delayed free recall scores [167] and the Craft story immediate and delayed recall scores [141]. The visual memory domain was the Brown Learning Test initial learning trials and delayed recall score [168]. The working memory score consisted of the Rao Paced Auditory Serial Addition Test (PASAT) 2 and 3 minute trial totals [144]. The processing speed score comprised the WAIS-III Digit Symbol-Coding score [143], the left- and right-hand grooved pegboard times [149], completion times from D-KEFS Trail Making Test trials 1-5, and the D-KEFS Color-Word Interference Test color naming, word reading, and color-word interference trials. The sorting domain was the D-KEFS Sorting Test number of correct sorts and the free sorting and

recognition trial description scores. The distractibility domain was the Gordon Continuous Performance Task (CPT) distractibility trial total correct and false positive scores, while the reaction time domain was the average of the distractibility and vigilance trials reaction times. The global score was the average of all the above domains as well as the WASI Block Design score.

Statistical Analyses

One-way ANOVA with Tukey post-hoc tests were used to examine between-group effects of age, education, IQ estimates, and BL-M1 interval, while chi-square examined handedness distribution. Experimental measures, including fMRI activation and deactivation, neuropsychological tests, 3-back task performance and reaction times, and depression and anxiety scores were assessed using a repeated measures general linear model in SPSS 19 with factors of group and time. Age covariates were used in imaging analyses, while neuropsychological measures were pre-adjusted for age. Group-by-time interactions from these analyses were examined, as well as main effects of group and time. Absolute values of activation and deactivation were summed at each time point to create a measure of total change in neural activity and analyzed using the same repeated measures model. Pearson correlations were used to assess relationships between changes in imaging variables and neuropsychological values.

Results

Demographics, psychological state, hormonal status and cancer treatment data are provided in Table 2-1. As expected, groups differed in age, with both post-menopausal groups significantly older than both pre-menopausal groups at baseline (overall ANOVA $p<0.001$; post-hoc Tukey tests $p<0.05$)

Education, handedness, estimated IQ, and BL-M1 interval did not differ between groups. CES-D and STAI-S mean scores were well below clinical thresholds and did not show main effects of group or time or group-by-time interactions. Several

cancer patients were using psychiatric medications at each study visit, with little change in medication regimens between BL and M1. Cancer stage and CTx regimens were similar between groups.

All post-menopausal HC and CTx participants reported amenorrhea >6 months at study entry, and all pre-menopausal HC had regular periods at study entry (Table 2-1). In the CIA group, 4 patients reported regular periods and 3 patients reported irregular periods at study entry, and all of these reported amenorrhea >6 months related to CTx at M1. Menstrual status was unavailable for 2 CIA patients, but they were placed in this group based on their ages of 44 and 45 at study entry, as all HC under 50 in this cohort were pre-menopausal. For hormonal medications, several post-menopausal patients reported using hormonal replacement therapy at each visit (4 at BL, 2 at M1). One 55-year-old CIA participant (the oldest in this group) was using estrogen for perimenopausal symptoms at BL but not at M1. No cancer patients were using antiestrogen treatments at BL, but 2 patients in each cancer group had started these treatments at M1.

Figure 2-1 depicts the activation and deactivation regions of interest used to derive values for each participant at BL and M1. Figure 2-2a shows mean values of activation and deactivation for each group at BL and M1. Repeated measures ANOVA with an age covariate did not show any significant main effects or interactions for either activation or deactivation. Repeated measures ANOVA on the summed magnitudes of activation and deactivation for the four groups with an age covariate revealed a significant group-by-time interaction ($p=0.006$). In contrast to the other groups, the CIA group showed an increase in the summed magnitudes of activation and deactivation from BL to M1 (Figures 2-2b, 2-2c), and this change was statistically significant ($p=0.011$). There was also a significant effect of time ($p=0.046$), with an overall pattern of increased magnitudes from BL to M1 and a time-by-age interaction ($p=0.047$) in which

younger participants tended to increase in magnitude over time (driven by the CIA group).

Figure 2-1 Working memory-related activation (3-back > 0-back contrast)) (a) and deactivation(0-back > 3-back contrast) (b) regions of interest derived from main effects of all groups' baseline and one month post-chemotherapy scans; p_{crit}=0.05, minimum cluster extent (k)=10.

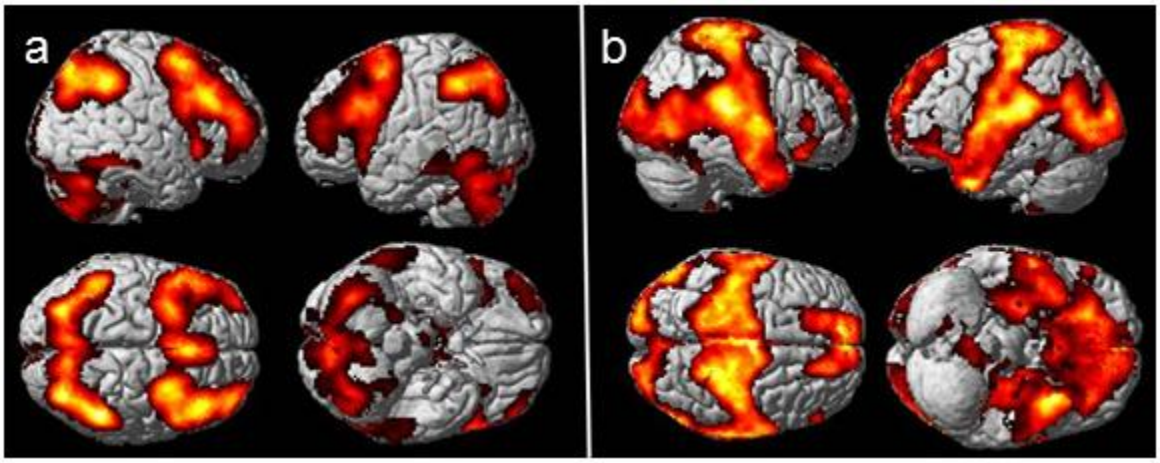


Figure 2-2a Working memory-related total activation and deactivation at baseline (BL) and 1 month post-chemotherapy (M1) (arbitrary units; mean±SD).

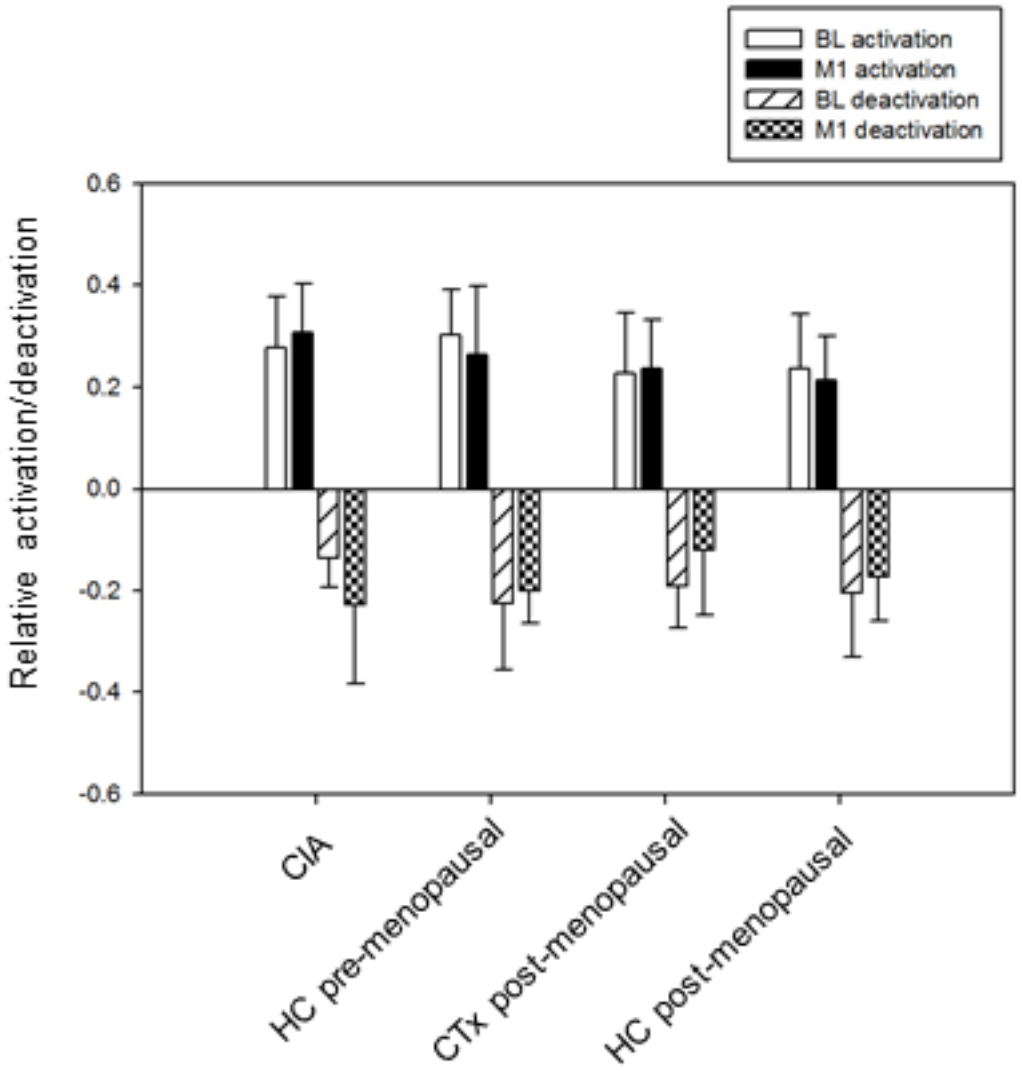


Figure 2-2b Summed magnitudes of activation and deactivation at BL and M1; group-by-time interaction is statistically significant (p=0.006), with only the CIA group showing change over time (p=0.011).

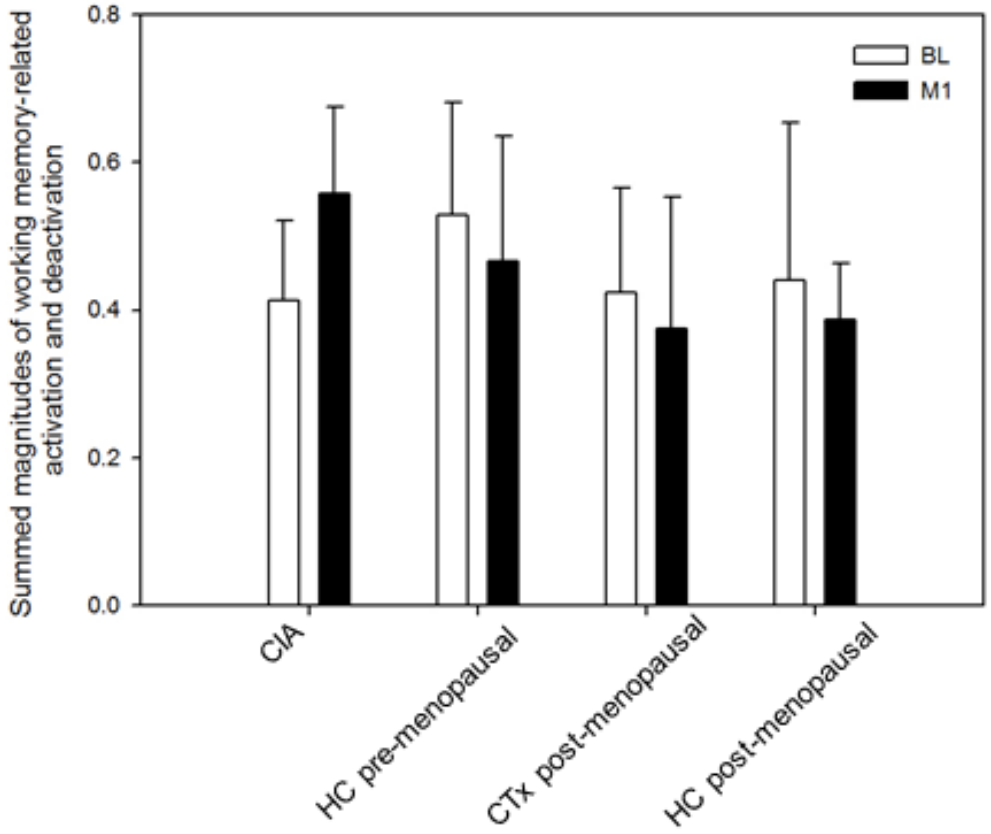
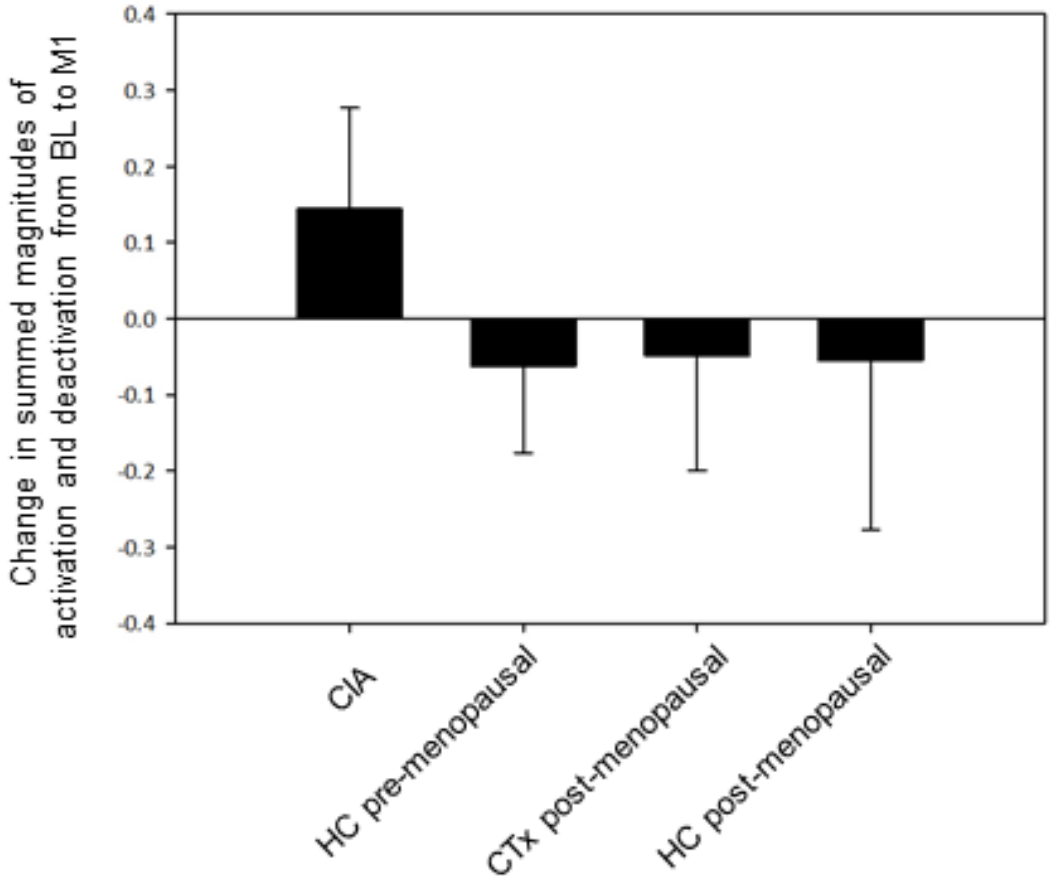


Figure 2-2c Change between BL and M1 in summed magnitudes of activation and deactivation.



In-scanner 3-back task performance and reaction times did not differ between groups or over time (Table 2-2).

Group means of neuropsychological testing domains are shown in Table 2-2. A main effect of time was present for most domains at $p \leq 0.01$.This reflected an overall tendency toward improved performance at M1, likely due to practice effects. Main effect of group was not statistically significant in any domain. Interestingly, trends toward group-by-time interactions were present in the verbal ($p=0.073$) and visual memory ($p=0.098$) domains. In both of these domains, all groups showed improved performance from BL to M1 except for the CTx post-menopausal group, whose performance decreased between sessions.

Table 2-2 Neurocognitive testing and in-scanner task results

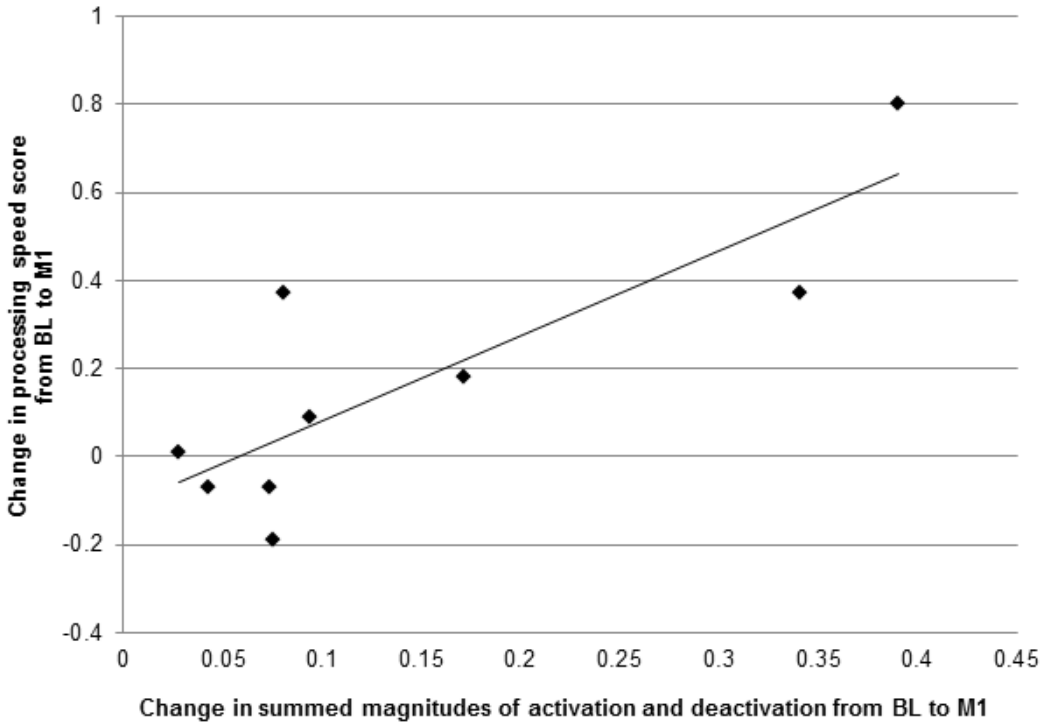
		Chemotherapy-induced amenorrhea (n=9)	**Healthy control pre-menopausal (n=6)**	**Chemotherapy post-menopausal (n=9)**	**Healthy control post-menopausal (n=6)**
Neurocognitive Testing Domains					
Verbal^	BL	-0.339±0.837	0.211±0.581	0.173±1.074	-0.264±0.976
	M1	-0.230±0.722	0.370±0.662	0.029±0.956	-0.029±0.723
Verbal Memory	BL	-0.271±1.029	-0.335±0.723	-0.345±0.932	0.380±0.767
	M1	-0.311±0.881	-0.092±0.495	-0.198±0.893	0.297±1.011
Visual Memory**^	BL	-0.241±1.42	0.179±0.607	0.222±0.549	0.145±1.336
	M1	0.584±1.548	0.848±0.778	0.135±0.618	1.071±1.615
Working Memory**	BL	0.125±0.965	0.019±0.290	0.778±0.718	0.123±0.977
	M1	0.659±1.157	0.421±0.585	1.091±0.920	0.312±0.670
Processing Speed*	BL	0.123±0.940	0.132±0.415	0.061±0.405	0.138±0.401
	M1	0.292±0.981	0.355±0.384	0.064±0.638	0.255±0.232
Sorting**	BL	-0.598±0.878	-0.035±0.912	-0.360±0.988	0.135±0.827
	M1	-0.011±1.079	0.613±0.659	-0.431±1.168	0.566±1.345
Distractibility**	BL	-1.276±1.940	-0.430±0.750	-0.475±2.213	0.468±1.342
	M1	-1.808±2.296	-0.438±0.651	-1.318±3.702	0.812±0.531
Reaction Time**	BL	0.319±0.701	-0.446±1.173	0.021±0.788	0.113±0.710

	M1	0.552±0.508	-0.097±1.318	0.291±0.637	0.407±0.628
Global**	BL	-0.320±0.760	-0.079±0.518	-0.076±0.713	0.190±0.710
	M1	-0.102±0.863	0.240±0.439	-0.096±0.982	0.447±0.569
In-scanner 3-back task					
3-back score (% corrected for guessing)	BL	65.3±27.3	74.4±7.8	62.1±24.6	78.5±16.1
	M1	65.3±27.3	74.3±12.7	63.2±25.0	67.1±12.7
Reaction time (seconds)	BL	1.20±0.31	1.01±0.13	1.01±0.20	1.15±0.10
	M1	1.19±0.34	0.97±0.10	1.09±0.15	1.16±0.08

** main effect of time $p \leq 0.01$
* main effect of time $p \leq 0.05$
^ group-by-time interaction trend $p < 0.10$

To assess the functional significance of the increased magnitude of neural activity in CIA, we tested for correlations between the activity change in this group and change in neuropsychological domain scores. A significant positive correlation emerged in the processing speed domain ($r=0.837$, $p=0.005$, survives Bonferroni threshold for 9 domains ($p=0.045$ after correction; Figure 2-3). The positive valence indicates that increased magnitude of neural activation/deactivation between BL and M1 is associated with improvement in processing speed scores. No other CIA activity change-neurocognitive domain correlations were statistically significant.

Figure 2-3 Correlation of change in summed magnitude of activation and deactivation with change in processing speed neuropsychological testing domain scores from baseline to one month post-chemotherapy in chemotherapy-induced amenorrhea (CIA) (r=0.837, p=0.045 after Bonferroni correction for 9 neuropsychological domains).



Discussion

Our results demonstrate prospectively that the pattern of change in brain activity from pre- to post-CTx varies according to pre-treatment menopausal status. BC patients who underwent CIA showed increased magnitudes of activation and deactivation from pre- to post-CTx, while CTx post-menopausal and both HC groups did not. In the context of maintained 3-back task performance, this may indicate effective compensatory neural activity in the CIA group. Age, an inevitable between-group confound in this type of analysis, was included as a covariate, suggesting that these effects are due to CIA itself. We found no evidence for the negative cognitive effect of CIA that has been observed in some studies [109, 110] but not others [111-113].

Relationships between brain changes and behavioral measures increase the potential clinical significance of our findings. CIA-related change in neural activity was highly correlated with improvement in neurocognitive processing speed scores from BL to M1, suggesting that this adaptation is functional. Processing speed, in addition to working memory and executive function, is one of the domains most often implicated in cancer- and treatment-related cognitive dysfunction [6].

The current results are consistent with the interpretation that the neural stress of CIA's abrupt decrease in estrogen, in addition to CTx itself, requires alterations in brain activity to maintain cognitive function. In light of evidence that both activation [28] and deactivation [29] increase parametrically with task demand, these results suggest that after CTx, the CIA brain responds to the same 3-back task as if it is more difficult. A similar phenomenon of effective compensatory activation has been observed in mild cognitive impairment (MCI) in older adults: in the early, higher functioning stages of MCI, patients show hippocampal and prefrontal hyperactivation during memory tasks compared to cognitively normal older adults, while activation decreases in later stages of MCI as cognitive functioning declines [31, 32].

Interestingly, in neuropsychological testing, the post-menopausal CTx group was unable to maintain performance in the post-CTx visit, while in all other groups scores improved between BL and M1 in the verbal and visual memory domains (in non-significant trends for group-by-time interactions). These domains involve different neural circuitry than the working memory scanner task and have not been especially previously implicated in CTx-related cognitive dysfunction, but the lack of increase in neural activity in the post-menopausal CTx group may be related to this decreased neurocognitive performance post-treatment. As estrogen loss reduces neuroplasticity, and longer duration of estrogen deprivation (i.e., more time after menopause) is associated with worse outcomes [57], the lack of change in brain activity may be related to reduced neuroplastic adaptation to CTx in the post-menopausal brain. This result is also consistent with a recent longitudinal study [159] which found that older age was related to increased risk for cognitive dysfunction after CTx, particularly in the context of processing speed domain and lower cognitive reserve (although no effects of pre-treatment menopausal status were observed). It should be noted, however, that the current study represents a small sample size relative to most cognitive studies, and the statistical effects are consequently modest. Follow-up in larger cohorts is warranted.

While there are no neuroimaging studies of CIA with which to compare the current results, neuroimaging studies of both cancer/CTx and estrogen suppression in pre-menopausal women do exist. In cancer and CTx, BC patients have shown pre-treatment frontal hyperactivation [44, 45] that is attenuated one month post-CTx but remains hyperactive one year later [45]. Task performance tended to decrease along with the attenuation of hyperactivation at one month and return to higher levels one year later [45]. Studies of longer-term survivors with a history of CTx have demonstrated lower activation in task-related regions [47, 49]. Prospective studies of pre-menopausal women who undergo abrupt estrogen suppression with gonadotropin releasing hormone (GnRH) agonists have shown globally attenuated task-related blood flow on PET in the context of

maintained task performance [94], and decreased frontal fMRI activation during memory encoding with a trend toward worse performance on the subsequent recognition test [92]. It is notable that in the GnRH agonist studies, estrogen is still being suppressed at the time of scanning, while at M1 in the current study, ovarian function may be starting to resume after CIA and several patients were using tamoxifen, which also affects estrogen function. All of the above studies measured regionally specific task-related activation, while the current study focuses on global levels of activation and deactivation, so direct comparison is difficult.

The effects of cancer and CTx on cognition and brain activity likely involve multiple biological pathways [10], and the present results contribute to a better understanding of one key pathway, hormonal changes. Possible mechanisms for CTx-related cognitive dysfunction, including oxidative stress and genetic factors, overlap with those for those thought to be involved in the deleterious effects of estrogen loss [10, 169, 170], suggesting possible compounding effects.

This study has several strengths. To our knowledge, this is the first neuroimaging study of CIA. The study is prospective, increasing confidence in attribution of brain effects to CIA. Two groups of HC were employed to match the CIA and post-menopausal CTx groups. Our analyses employed either an age covariate or data pre-adjusted for age, minimizing the effects of this potential confound. Comprehensive neuropsychological testing was conducted in order to determine domain-specific effects of treatment. Finally, the strong correlation in CIA between changes in neural activity and processing speed is promising for the behavioral relevance of these results.

This study also has several limitations. Pre- and peri-menopausal BC patients were included in the CIA group, potentially increasing variability. Several of our BC patients used antidepressants and post-CTx anti-estrogen therapy, which is difficult to avoid in this population. We relied on self-reported menstrual status at

each session rather than hormone levels; future work correlating cognitive and imaging variables with hormone levels would be informative.

Subsequent studies should follow the effects of CIA longitudinally. While 80% of pre-and peri-menopausal BC patients experience CIA in the months immediately following CTx [102-105], only 20-60% remain amenorrheic 6-12 months later [105, 114, 115]. Complicating matters, tamoxifen has been shown to potentiate CIA [115], and post-menopausal CTx comparison groups will likely be using aromatase inhibitors which may have different neural effects than tamoxifen. While estrogen is neurotrophic and neuroprotective in vitro, the effects of estrogen loss (and replacement) on the human brain are complicated and controversial [62, 114], and CIA is no exception. Despite these complications, it will be important to determine the neural effects of CIA throughout survivorship. Other types of fMRI analyses (voxelwise, resting state, etc.) will also be informative, as estrogen influences both structural and functional connectivity [171]. While BC patients cannot be treated with any agent that is an estrogen agonist to breast tissue, future intervention with other agents, such as specially designed “neuro-SERMs” [127, 172], which might be estrogen antagonists to breast and uterus and estrogen agonists to brain and bone, may prove beneficial. The current findings have implications for risk appraisal and development of prevention or intervention strategies for cognitive changes in CIA.

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Chapter 3: *ESR1* variation and brain structure in cognitively normal older adults: effects of sex, brain region, and *APOE*-ε4 allele

Neural effects of estrogen are thought to contribute to sex differences in prevalence and presentation of psychiatric and neurological disorders. Variation in *ESR1* (coding for estrogen receptor-α (ER-α)), particularly in the single nucleotide polymorphisms (SNPs) rs2234693 and rs9340799, has been found to be related to many of these disorders, but little work has focused on its relationship to brain structure. We examined MRI scans and *ESR1* genotypes from 195 cognitively and psychiatrically healthy older adults recruited as part of the Alzheimer's Disease Neuroimaging Initiative. Whole-brain voxel-based morphometry, cortical thickness analyses, and automated medial temporal volumetry were used to probe brain-genotype relationships. Voxel-based morphometry revealed an interaction in which males carrying a T allele of rs2234693 or an A allele of rs9340799 showed greater gray matter (GM) density in regions distributed throughout the brain, while females carrying a C allele of rs2234693 or a G allele of rs9340799 showed greater GM density. Cortical thickness analysis showed similar results. These effects were not modulated by *APOE*-ε4 carrier status. Right amygdala volumes, with trends for left amygdala and left hippocampus, were significantly greater in females with the rs9340799 GG genotype compared to the AA genotype. Furthermore, in females, *APOE*-ε4 carrier status interacted with rs2234693 genotype with regard to amygdala volumes. This study is the first to demonstrate that, even in cognitively and psychiatrically normal older adults, genetic variation in estrogen receptors affects brain morphology in ways that differ by sex, brain region and *APOE*-ε4 carrier status.

Introduction

Neural effects of estrogen are thought to contribute to sex differences in prevalence and presentation of psychiatric disorders, including depression [173], schizophrenia [174], and others [175], and neurological disorders such as Parkinson's disease [51] and Alzheimer's disease (AD) [52, 176, 177]. Estrogen is critically involved in memory and affective functions [175]. On a cellular level, estrogen is neuroprotective, interacting with a variety of neuroplastic processes [57], growth factors [64], and neurotransmitters [65]. Estrogen also influences pathways important in AD pathogenesis, including amyloid, tau, and acetylcholine synthesis [63, 67-69, 178]. While most circulating estrogen in premenopausal females is produced by the ovaries, estrogen is produced throughout life in both sexes by neurons, astrocytes, and cerebrovascular endothelial cells [65]. In men, brain aromatase converts circulating testosterone to estrogen, which is important in cognition [50]. Neural estrogen receptors are widely expressed in both sexes.

Estrogen receptor genetic variation can alter the downstream neural effects of estrogen, and relationships between this variation and clinical phenotypes have been well documented. Polymorphisms in *ESR1*, the gene coding for estrogen receptor-α (ER-α), have been associated with a number of psychiatric disorders (see [79] for review), including postmenopausal depression [179], premenstrual dysphoric disorder [180], methamphetamine-induced psychosis in men [181], childhood-onset mood disorders in girls [182], and obsessive-compulsive disorder [183] and schizophrenia in both sexes [184]. *ESR1* variation has been associated with age of onset or risk of AD in both sexes [71, 185-188], and *ESR1* genotype may interact with the apolipoprotein E-ε4 (*APOE*-ε4) allele in influencing AD onset [71-73]. Further, in healthy individuals, *ESR1* polymorphisms have also been associated with cognitive function [70] and personality traits [189]. Two *ESR1* Intron 1 single nucleotide polymorphisms (SNPs) rs2234693, previously called PvuII, and rs9340799, or XbaI, have been

associated with a wide range of clinical phenotypes, and a considerable number of *ESR1* studies referenced above include these SNPs.

Despite evidence for *ESR1* involvement in many CNS disorders, only one study has assessed its relationship to brain phenotypes. den Heijer and colleagues [190] studied 468 older adults from the Rotterdam Scan Study, including 25 who developed dementia. Manually segmented amygdala volumes were significantly associated with rs2234693 and rs9340799 genotype in women but not men, regardless of dementia status, with similar trends for hippocampal volumes. These medial temporal lobe (MTL) structures are implicated in numerous cognitive and psychiatric disorders.

To investigate structural changes throughout the brain associated with *ESR1* variation, we examined MRI scans from 195 cognitively and psychiatrically healthy older adults in the Alzheimer's Disease Neuroimaging Initiative (ADNI) cohort. The current study aimed to: 1) determine, in each sex, the relationship of rs2234693 and rs9340799 genotype to gray matter (GM) density throughout the brain and automatically traced MTL volumes; 2) examine the relationship of these SNPs to cortical thickness; 3) assess the role of *APOE*-ε4 allele on these phenotypes

Methods

Sample

Data were obtained from the ADNI database (http://www.loni.ucla.edu/ADNI). Data downloaded for 228 cognitively normal participants included baseline 1.5 T MRI scans, SNP genotypes, demographics, *APOE* genotype, and current medications. Exclusion criteria for ADNI included recent history of depression, bipolar disorder, substance abuse or dependence, and any history of schizophrenia. Females were at least two years past menopause or surgical sterilization.

MRI Processing

Participants had at least two 1.5T MP-RAGE scans at baseline according to the ADNI MRI protocol [191]. As described elsewhere [192], each scan was independently processed using two automated techniques: whole-brain voxel-based morphometry (VBM) [193-195] and segmentation and parcellation using FreeSurfer v. 4.01 [196, 197] (http://surfer.nmr.mgh.harvard.edu/). Briefly, image processing utilized SPM5 (http://www.fil.ion.ucl.ac.uk/spm/) to create GM density maps ($1mm^3$ voxels, smoothed with a 10mm FWHM Gaussian kernel) normalized to Montreal Neurological Institute (MNI) atlas space. Two independent, smoothed, normalized GM density maps for each participant were averaged to create a mean map. Two participants failed VBM segmentation. FreeSurfer was used to automatically identify cortical and subcortical tissue classes using an atlas-based Bayesian segmentation procedure. Cortical thickness values, MTL structure volumes, and total intracranial volume (ICV) were extracted for all participants. FreeSurfer values from the two MP-RAGE images obtained at the same study visit for each participant were averaged to create mean values. Amygdala and hippocampus volumes were adjusted for age and ICV. Cortical thickness values were registered to the cortical surface of a template created from all ADNI healthy control participants.

ESR1 Genotyping

Genotyping and quality control were completed using the Illumina Human601-Quad BeadChip, Illumina BeadStudio 3.2 software (Illumina, Inc., San Diego CA), and PLINK v1.06 software [198] (http://pngu.mgh.harvard.edu/purcell/plink/) in a protocol described elsewhere [199, 200]. Population stratification analysis suggested the advisability of restricting analyses to non-Hispanic Caucasians. After the QC procedure, 203 of the 226 participants with usable MRI data remained.

As *ESR1* rs2234693 and rs9340799 were not included among SNPs genotyped on this chip, they were imputed using MaCH [201]

(http://www.sph.umich.edu/csg/yli/mach/index.html) and the HapMap2 release 24 CEU reference sample (individuals of Northern and Western European ancestry) [202] (http://hapmap.ncbi.nlm.nih.gov/index.html.en). Imputed genotypes with posterior probability for the most likely genotype less than 0.9 were excluded. 195 participants remained for rs2234693 and 178 for rs9340799. PLINK v.1.07 was used to calculate linkage disequilibrium between the two SNPs in the study population. rs2234693 (PvuII) has a T (p) major allele and a C (P) minor allele; rs9340799 (XbaI) has an A (x) major allele and a G (X) minor allele. We will refer to the SNPs using rs numbers and allele nucleotides rather than the older PvuII and XbaI nomenclature.

VBM Statistical Analyses

Voxelwise statistical analyses were performed using SPM5. For each SNP an omnibus group ANOVA was constructed with factors of sex and genotype (TT, CT, or CC for rs2234693; AA, GA, or GG for rs9340799), including age and ICV as covariates. A voxel-level critical significance threshold (p_{crit}) of 0.005 was used. With this p_{crit}, a minimum cluster extent (k) was used to retain only regions that survived an uncorrected cluster-level threshold of $p \leq 0.05$. Both voxel-level and cluster-level significance values are reported to address multiple comparisons. Voxel-level significance values represent the probability (under the null hypothesis) of finding a voxel with as great or greater a peak statistical threshold (T). Uncorrected cluster-level significance values ($p_{uncorrected}$) represent the probability (under the null hypothesis) of finding a cluster with as great or greater a number of voxels (k). Correction for whole-brain search volume results in corrected cluster-level significance values ($p_{corrected}$).

Within the omnibus ANOVA, weighted contrast vectors were used to create contrast maps showing voxels in which local GM density differed between groups. Mean GM density values for each subject were extracted from significant clusters using the MarsBaR SPM toolbox [165] (http://marsbar.sourceforge.net).

Cortical Thickness: SurfStat

SurfStat [203] (http://www.math.mcgill.ca/keith/surfstat/) was used to determine areas of group difference in cortical thickness, using the same omnibus ANOVA and weighted contrast vectors described above for VBM analyses. Correction for multiple comparisons used the random field theory (RFT) method at a 0.05 level of significance.

Other Statistical Analyses

Demographic, MTL volume, and *APOE*-ε4 epistasis analyses were performed in PASW Statistics v18.0 (SPSS, Inc., Chicago IL) separately for rs2234693 and rs9340799. For most demographic information and all MTL volumes, males and females were analyzed separately using one-way ANOVA with a factor of genotype; between-sex demographic differences were determined using one-way ANOVA with a factor of sex. Results were considered significant at $p \leq 0.05$, and post-hoc Tukey tests were used for pairwise comparisons. Chi-square tests were used to compare handedness and *APOE*-ε4 carrier status among genotypes within each sex as well as genotype distributions, handedness, and *APOE*-ε4 carrier status between sexes. Multivariate general linear model analysis was used to examine the interaction of *APOE*-ε4 carrier status and *ESR1* genotype on brain phenotypes. Factors were *ESR1* genotype and *APOE*-ε4 carrier status (ε4 present or absent) and dependent variables were mean GM cluster values (from VBM) or MTL volumes. To determine the amount of variance accounted for by genotype (r^2), linear regressions with genotype (number of minor alleles: 0, 1, or 2) were performed for mean cluster values from VBM analyses and for MTL volumes.

Results

Participant Characteristics and Genotypes

Distribution of genotypes between sexes was not significantly different for either SNP (chi-square $p>0.05$). Table 3-1 includes demographic information by sex and genotype for each SNP. Males with CC (rs2234693) or GG (rs9340799)

genotypes showed significantly, although not clinically meaningfully, higher Geriatric Depression Scale (GDS) [204] scores *(p*<0.05). Mini-mental status examination (MMSE) [205] scores and Neuropsychiatric Inventory Questionnaire (NPI-Q) [206] scores did not differ between genotypes. Educational attainment varied between sexes for both SNPs (*p*<0.001); thus, VBM analyses (which included a factor of sex in the omnibus ANOVA) were repeated using an education covariate, with the overall pattern of results unchanged.

Table 3-1 Demographic, cognitive, and psychiatric assessment of participants for rs2234693 and rs9340799 separated by sex and genotype. Mini Mental Status Exam, Geriatric Depression Scale, and Neuropsychiatric Inventory Questionnaire scores were all adjusted for age. P-values represent one-way ANOVA by genotype for each sex, except for *APOE*-ε4 carrier status and handedness, which represent chi-square tests. Asterisks denote significant effects of genotype (p<0.05).

rs2234693 (Pvull)									
Gender	*Male*				*Female*				
Genotype	TT (pp)	CT (Pp)	CC (PP)	p-value	TT (pp)	CT (Pp)	CC (PP)	p-value	Between-gender p value
N	35	51	19		22	47	21		
APOE-ε4 carriers (%)	9(26)	14(27)	5(26)	0.983	6(27)	15(32)	4(19)	0.548	0.494
Handedness R:L	31:4	47:4	17:2	0.845	20:2	45:2	20:1	0.704	0.220
Age	74.42(0.82)	76.66(0.73)	77.24(1.40)	0.073	77.68(1.23)	76.00(0.63)	76.07(0.48)	0.079	0.977
Education (years)	17.29(0.34)	16.45(0.41)	17.11(0.59)	0.309	14.41(0.48)	15.51(0.40)	15.48(0.63)	0.250	<0.001
Mini Mental Status Exam	29.01(0.16)	28.95(0.16)	29.27(0.19)	0.502	29.24(0.21)	29.23(0.12)	29.46(0.16)	0.561	0.059
Geriatric Depression Scale	0.74(0.17)	0.68(0.16)	1.50(1.29)	0.024*	0.74(0.18)	0.96(0.19)	0.79(0.20)	0.713	0.908
Neuropsychiatric Inventory Questionnaire	0.43(0.19)	0.33(0.15)	0.47(0.28)	0.868	0.23(0.11)	0.21(0.07)	0.62(0.27)	0.102	0.566
rs9340799 (Xbal)									
Gender	*Male*				*Female*				
Genotype	AA (xx)	GA (Xx)	GG (XX)	p-value	AA (xx)	GA (Xx)	GG (XX)	p-value	Between-gender p value
N	41	48	9		33	38	9		
APOE e4 carriers (%)	11(27)	12(25)	3(33)	0.872	9(27)	12(32)	2(22)	0.831	0.435
Handedness R:L	36:5	45:3	8:1	0.612	30:3	37:1	9:0	0.353	0.220
Age	74.87(0.78)	77.26(0.77)	77.72(2.00)	0.077	77.17(1.00)	75.51(0.64)	75.31(1.51)	0.304	0.864
Education (years)	17.10(0.31)	16.75(0.44)	17.00(1.00)	0.822	14.79(0.41)	15.26(0.44)	15.11(1.02)	0.704	<0.001
Mini Mental Status Exam	29.09(0.14)	28.97(0.15)	29.35(0.24)	0.540	29.28(0.16)	29.28(0.13)	29.10(0.31)	0.846	0.131
Geriatric Depression Scale	0.90(0.20)	0.60(0.14)	1.75(0.52)	0.023*	0.80(0.17)	1.01(0.20)	0.46(0.34)	0.385	0.882
Neuropsychiatric Inventory Questionnaire	0.39(0.16)	0.33(0.16)	0.11(0.11)	0.759	0.24(0.09)	0.26(0.10)	0.57(0.56)	0.523	0.720

To ensure that hormonal or psychoactive medication effects did not compromise results, all analyses were repeated excluding seven participants using the following agents: one male for finasteride (an antiandrogen), one female for estradiol, one female for gabapentin, and four females and one male for opiates. The overall pattern of results was unchanged after these exclusions. No participants were using antidepressants, anxiolytics, or antipsychotics.

The minor allele frequency was 0.46 for the rs2234693C allele and 0.34 for the rs9340799 G allele. Linkage disequilibrium (LD) analysis for the two SNPs indicated high values of r^2=0.741 and D'=1.00, and the CG and TA haplotypes were in phase (i.e., the two alleles are inherited together more frequently than expected under linkage equilibrium).

Gray Matter Density and Cortical Thickness

VBM results from rs9340799 will be discussed here, as genotype effects were stronger for this SNP. As expected, since the SNPs are in strong LD, the pattern of results was similar for rs2234693 (not shown). rs9340799 genotype had differential effects on GM density by sex: in a sex by genotype interaction, males carrying a major allele (A) and females carrying a minor allele (G) showed areas of increased GM density (Figure 3-1), including bilateral frontal, parietal, and temporal regions (Table 3-2). This "increased GM density in carriers" was the best fit of several models, including comparing homozygotes only (i.e., AA>GG and GG>AA) or increased density in homozygotes (i.e., AA>GA, GG and GG>GA, AA) (data not shown). Single-sex comparisons revealed that, in general, these effects were more prominent in frontal regions in males and parietal regions in females. The opposite contrasts (greater GM density in males carrying a G or in females carrying an A) did not show significant clusters. Cortical thickness analysis using these contrasts revealed similarly distributed results (Figure 3-1), although the female-only contrast did not show any significant clusters.

Figure 3-1 Surface renderings of statistically significant clusters in a voxel-based morphometry analysis of gray matter density (left) or a SurfStat analysis of cortical thickness (right) for rs9340799. Clusters significant in a genotype-by-sex interaction are shown in the top row, with clusters from both within-sex components of that interaction below. For gray matter density, voxel-level p_{crit} was set to 0.005 and a minimum cluster extent of k=1900 was used to retain only clusters with $p_{uncorrected} \leq 0.05$. For cortical thickness, a Random Field Theory correction method was used at a 0.05 significance level.

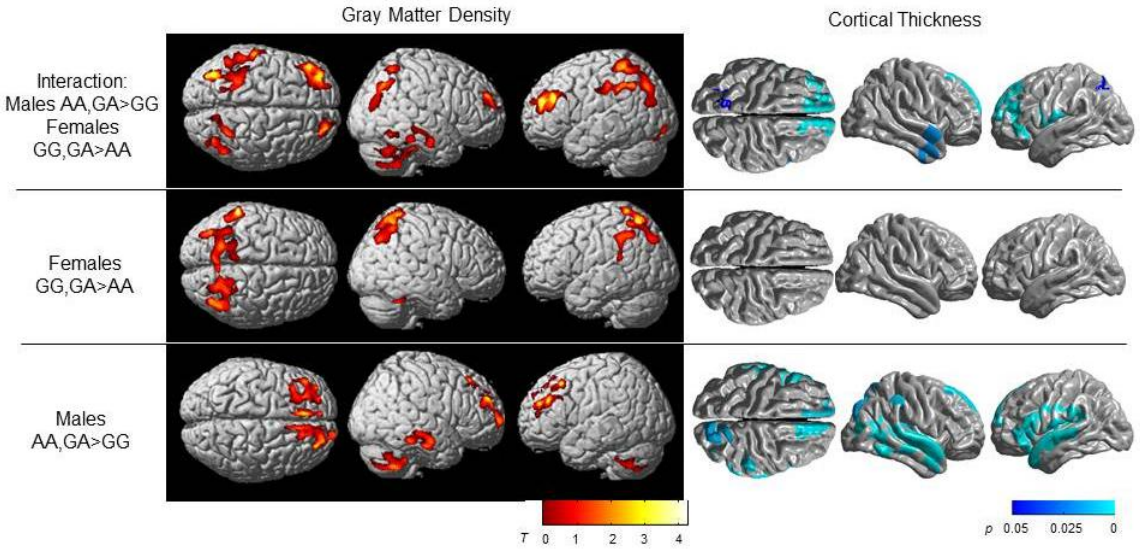


Table 3-2 Description of statistically significant clusters derived from voxel-based morphometry omnibus ANOVA including factors of *ESR1* rs9340799 genotype and sex. Clusters significant in a genotype-by-sex interaction are listed first, followed by clusters from both within-sex components of that interaction. Voxel-level p_{crit} was set to 0.005 and a minimum cluster extent of k=1900 was used to retain only clusters with $p_{uncorrected} \leq 0.05$. (No significant clusters were found using the opposite interaction or its within-sex components.)

Peak MNI coordinates (x y z)	Cluster extent (k)	Cluster-level $p_{corrected}$	Cluster-level $p_{uncorrected}$	Peak voxel-level $p_{uncorrected}$	T	Region description of local maxima within cluster	Brodmann Areas
rs9340799 Interaction: Males AA, GA>GG; Females GG, GA>AA							
38 -35 -24	20498	<0.001	<0.001	<0.001	4.52	Right fusiform and inferior temporal gyri	19,20
-8 -56 -17	9186	0.006	<0.001	<0.001	3.96	Left cerebellum, left parahippocampal and right posterior cingulate gyri	30,36
-26 48 23	6937	0.023	0.001	<0.001	3.92	Left superior and middle frontal gyri	10, 46
20 54 24	1926	0.688	0.054	<0.001	3.89	Right superior frontal gyrus	10
-62 -25 39	4125	0.156	0.008	<0.001	3.83	Left postcentral gyrus and inferior parietal lobule	2, 40
28 -55 58	2308	0.551	0.037	<0.001	3.65	Right superior parietal lobule and precuneus	7
-35 -74 38	6590	0.028	0.001	<0.001	3.62	Left precuneus and postcentral gyrus	2, 5, 19
-12 -79 0	4480	0.121	0.006	<0.001	3.60	Left lingual and middle occipital gyri	18
37 -69 40	3270	0.288	0.016	<0.001	3.38	Right precuneus, inferior parietal and middle temporal gyri	19,39,40
rs9340799: Males AA, GA>GG							
51 -21 -13	4181	0.150	0.008	<0.001	4.40	Right superior, middle, and inferior temporal gyri	20, 21, 22
-34 -8 -6	2157	0.604	0.043	<0.001	4.26	Left claustrum	N/A

20 53 25	6130	0.038	0.002	<0.001	3.92	Right superior and medial frontal gyri	8, 10
-23 47 23	5200	0.073	0.004	<0.001	3.86	Left superior and middle frontal gyri	8, 9, 10
35 -60 -37	7181	0.019	0.001	<0.001	3.76	Right cerebellum	N/A
-7 30 44	2140	0.610	0.044	<0.001	3.60	Left medial and superior frontal gyri	8
-34 -50 -42	2081	0.631	0.046	<0.001	3.43	Left cerebellum	N/A
rs9340799: Females GG, GA>AA							
-21 -61 62	7376	0.017	0.001	<0.001	4.23	Left superior parietal lobule and postcentral gyrus	5, 7
37 -37 -24	4167	0.151	0.008	<0.001	3.88	Right fusiform gyrus and cerebellum	20
-24 -34 -15	2348	0.538	0.036	<0.001	3.87	Left parahippocampal gyrus and cerebellum	36
22 -62 63	6993	0.022	0.001	<0.001	3.69	Right postcentral gyrus and superior and inferior parietal lobules	5, 7, 40
-57 -41 40	2929	0.366	0.021	<0.001	3.65	Left inferior parietal lobule and superior temporal gyrus	22, 40

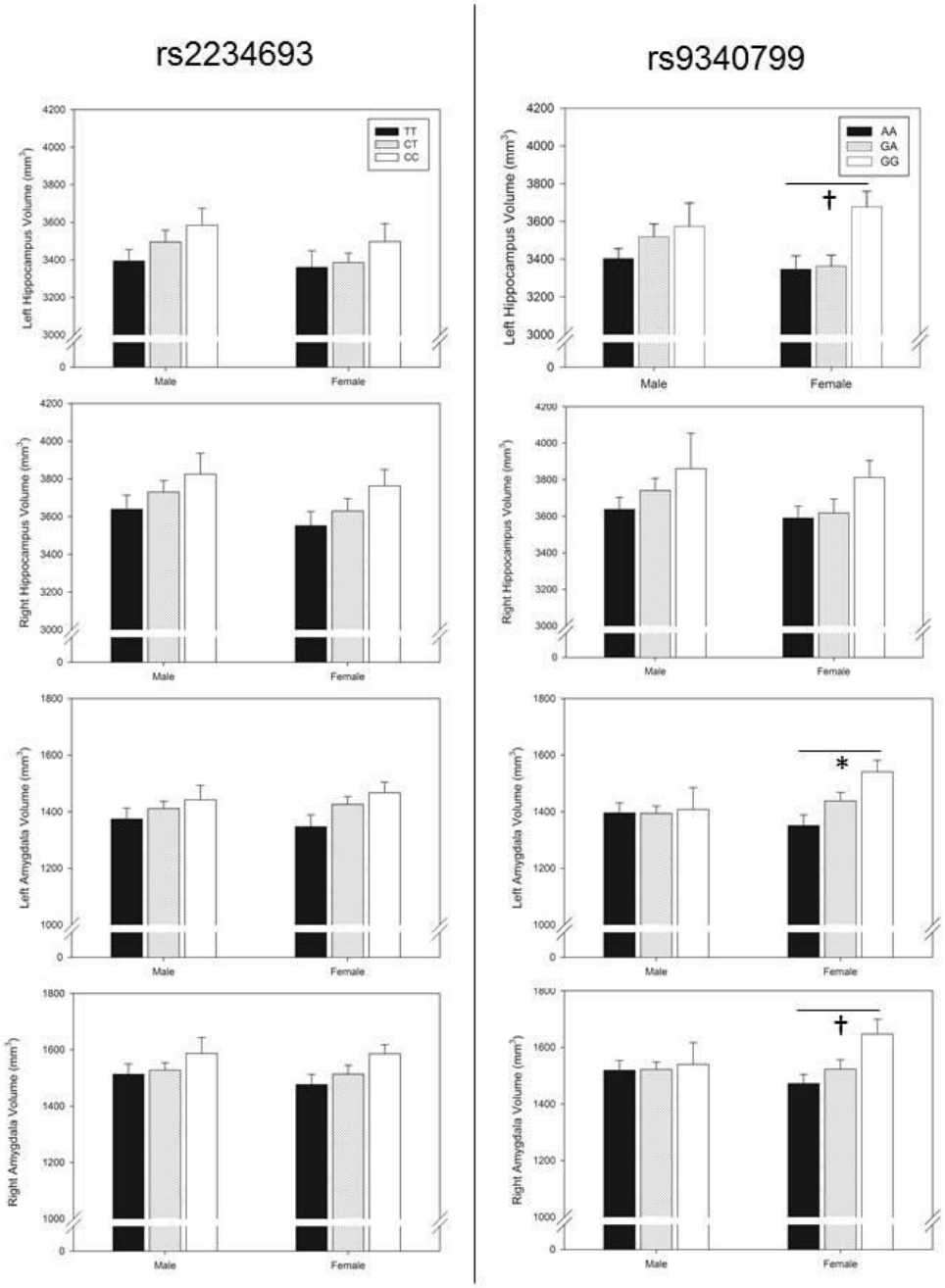
Using mean GM density values from clusters in the male-only VBM contrast, the variance accounted for by rs9340799 genotype (classified as 0, 1, or 2 minor alleles) ranged from 4-17%. Analyses in the female-only contrast showed that genotype accounted for 11-19% of variance.

Multivariate general linear models did not indicate epistasis between *ESR1* genotype and *APOE*-ε4 carrier status (i.e., no *ESR1* by *APOE* interactions) for any cluster from either sex-specific contrast for either SNP.

MTL Volumetry

Figure 3-2 shows group mean values for hippocampus and amygdala volumes. Similar to VBM analyses, significant genotype associations with volume were observed for rs9340799 but not rs2234693. In females a significant effect of rs9340799 genotype was found for the left amygdala (p=0.025). A post-hoc Tukey test revealed that female participants with a GG genotype showed greater volumes than those with an AA genotype ($p \leq 0.05$). Trend level results following the same pattern were seen for females for the right amygdala (p=0.057) and left hippocampus (p=0.056). In general, there was an overall pattern across sexes, structures, and SNPs of association of the minor allele with higher volumes.

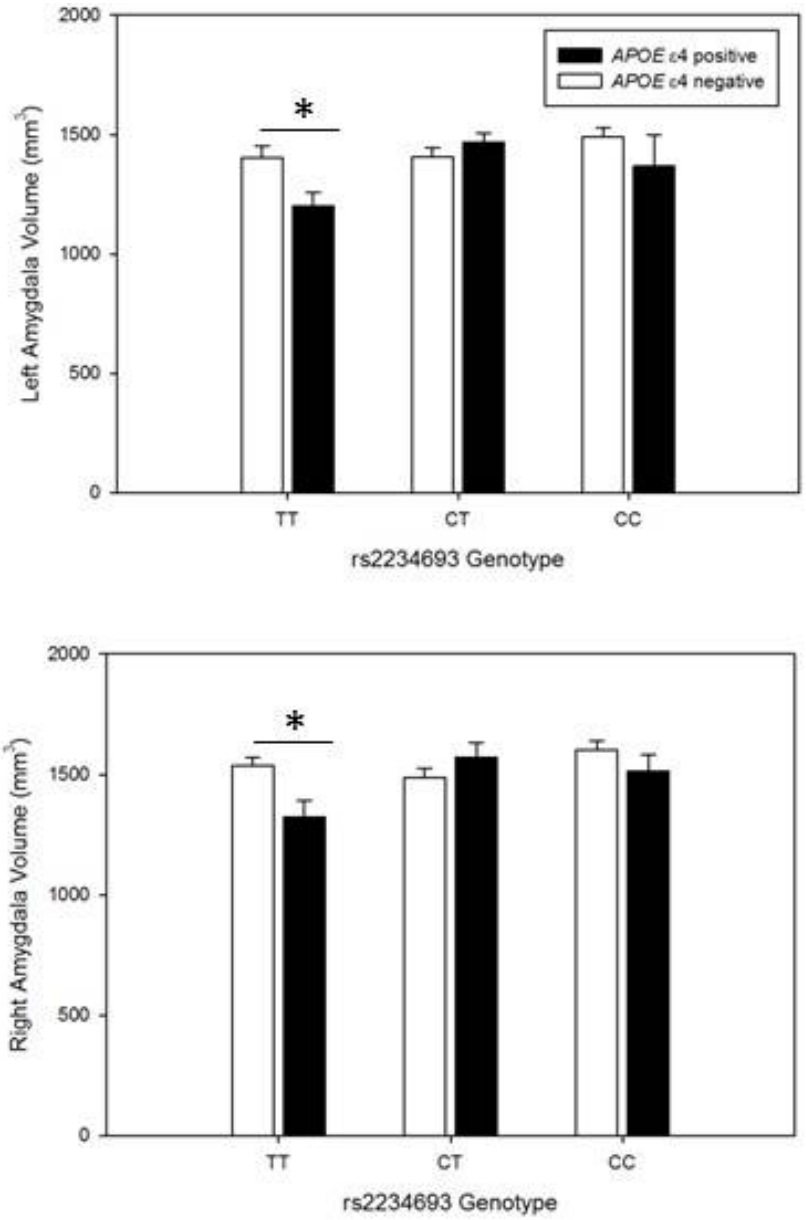
Figure 3-2 Mean age- and intracranial volume-adjusted bilateral hippocampus and amygdala volumes separated by sex and genotype for both rs2234693 (left column) and rs9340799 (right column). Bars represent mean ±SEM.*: $p<0.05$ in one-way ANOVA by genotype within gender. †: $p<0.06$ in one-way ANOVA by genotype within gender.



In structures with statistically significant or trend-level genotype effects (left amygdala, left hippocampus, and right amygdala volumes for rs9340799 in females) genotype accounted for 9.1%, 1.5%, and 6.5% of variance, respectively.

Epistasis between *ESR1* rs2234693 genotype and *APOE*-ε4 allele (i.e., *ESR1* genotype by *APOE* carrier status interaction) was observed for females but not males in both left (p=0.035) and right (p=0.017) amygdalae (Figure 3-3) Post-hoc t-tests revealed that in females of the rs2234693 TT genotype, but not CT or CC, *APOE*-ε4 carriers had significantly lower left (p=0.029) and right (p=0.005) amygdala volumes. For rs9340799 no interactions with *APOE* status were evident.

Figure 3-3 *ESR1* rs2234693 genotype by *APOE*-ε4 carrier status interaction in bilateral amygdala in women. Multivariate ANOVA with factors of rs2234963 genotype and *APOE*-ε4 status showed significant interaction ($p \leq 0.05$); asterisks represent $p \leq 0.05$ between *APOE*-ε4 carriers and non-carriers in post-hoc t-tests. Bars represent mean ±SEM. TT genotype n=22 (27% APOE- ε4 carriers), CT genotype n=47 (32% APOE- ε4 carriers), and CC genotype n=21 (19% APOE- ε4 carriers).



Discussion

This study is the first to demonstrate that genetic variation in *ESR1* has widely distributed effects on brain structure that differ by both sex and brain region. In our examination of 195 cognitively and psychiatrically normal older adults, there was greater GM density in regions throughout the brain in males carrying an rs9340799 A allele or an rs2234693 T allele (major alleles), while in contrast, GM density was greater in females carrying an rs9340799 G allele or an rs2234693 C allele (minor alleles). These effects on GM were not modulated by *APOE*-ε4 carrier status. Cortical thickness analyses showed similarly distributed results. We found in females that left amygdala volume, was significantly associated with rs9340799 genotype, with similar trends for right amygdala and left hippocampus volumes. Higher volumes in both sexes were observed with the GG genotype compared to AA the left amygdala, with consistent patterns of greater MTL volumes associated with minor alleles. These MTL volumetry findings are consistent with a previous study [190] in which women, but not men, with homozygous minor allele (CC or GG) genotypes showed increased amygdala volumes. Finally, *APOE*-ε4 carrier status modulated the effect of rs2234693 on amygdala volumes in women, with low volumes in *APOE*-ε4 carriers compared to non-*APOE*-ε4 carriers in the TT genotype only. This evidence of epistasis between *APOE* and *ESR1* in influencing brain structure has not been reported previously.

Sexual dimorphisms are well-documented in prevalence and presentation of many psychiatric and neurological disorders. While the neuropathophysiological mechanisms underlying these differences remain poorly understood, sex hormone signaling is thought to play an important role. This study is the first to demonstrate that, even in cognitively and psychiatrically healthy older adults, genetic variation in estrogen signaling widely affects brain morphology in ways that differ by sex and brain region. Notably, the affected areas observed in the present report, including MTL and prefrontal cortex, have been implicated in many psychiatric and neurological disorders. Our results document a differential

pattern of estrogen influence on brain morphology. In turn, this suggests that estrogen signaling is an important pathway in the study of sex differences in such disorders. An estrogen-dependent mechanism for the reported *ESR1*-associated morphological changes is highly plausible based on two areas of consistency with previous literature. First, the affected regions (hippocampus, amygdala, and distributed cortical areas [207], including the prefrontal cortex [208]), have been shown to express ER-α in both male and female humans and/or non-human primates, with the amygdala being particularly rich in ER-α expression [209]. Our volumetric results are also consistent with previously described ER-α expression patterns in showing greater *ESR1* effects in amygdala compared to hippocampus. Second, the brain regions associated with *ESR1* genotype in this report have been demonstrated to be estrogen-responsive in human structural neuroimaging studies, with higher-estrogen states generally associated with greater GM density in women of the same age. Specifically, when results are combined across studies comparing postmenopausal women using and not using hormone replacement therapy [81-85, 210], premenopausal women across various stages of the menstrual cycle [211, 212], and premenopausal women using and not using hormonal contraceptives [212], a total of 15 brain regions have been demonstrated to be estrogen-sensitive. Of these 15, 11 were found in our study, including prefrontal cortex, medial frontal gyrus, postcentral gyrus, fusiform, lingual, and parahippocampal gyri, hippocampus, inferior, middle, and superior temporal gyri, basal ganglia, and cerebellum. Overall, the considerable regional overlap of our results with previously described ER-α expression patterns and estrogen-sensitive regions is consistent with an estrogen-dependent mechanism for *ESR1*-related morphological differences, rather than *ESR1* variation serving as a proxy for some other unrelated marker.

The interaction by which genetic variation in *ESR1* is associated with opposite effects on GM density between sexes is particularly interesting. In the current study, the C and G minor alleles of the respective SNPs were associated with both greater MTL volumes in females (with similar patterns in males), The minor

C and G minor alleles was also associated with greater cortical GM density in females and decreased cortical GM density in males. Thus, the same *ESR1* genotype is associated with greater density/ volume of MTL in both sexes and cortex in females, with the opposite effect in male cortex. Supporting preclinical evidence suggests that estrogen exposure may differentially affect brain regions by sex at the cellular level: in adult female rats, exposure to estrogen increases spine synapses in both hippocampus and prefrontal cortex, whereas estrogen exposure in adult male rats increases spine synapses in prefrontal cortex but not hippocampus (for review, see [51]).

Numerous studies have identified the C and G minor alleles as risk factors for neurological or psychiatric disorders. However, the studies are conflicting and it is not clear how ethnicity and gender may modulate these effects [70]. In a meta-analysis of AD genetic association studies, Bertram and colleagues found that the C and G alleles are associated with AD, with odds ratios of ~1.2 [185]. Sundermann and colleagues found in a recent review that 8 of 18 studies identified C and G alleles as risk factors for cognitive impairment, and 2 of 4 studies identified C and G as risk alleles for mood and anxiety disorders [70]. Weickert et al. [184] found overtransmission of the C allele in schizophrenia patients. The association of the C and G “risk” alleles with lower GM density in men observed in the current report is consistent with these previous studies. Further, the association of the G “risk” genotype with greater amygdala volumes in women is interesting in light of evidence for greater amygdala volumes in depressed patients [213]. It should be noted that the greater cortical GM density in women with the “risk” alleles may be related to the postmenopausal status and lack of current hormone replacement therapy in the study population, or to other factors. Future work will be necessary to comprehensively identify *ESR1* genotype-by-sex interactions in the substrates of neurological and psychiatric disorders.

The mechanism by which rs2234693 and rs9340799 affect neural estrogen signaling is unclear. In the brain, estrogen acts at both organizational (developmental) and activational (exposure later in life) levels; genetic variation in ER-α could influence both. In vitro results suggest that the C but not T genotype of rs2234693 allows binding of the transcription factor b-Myb to the ER-α protein, with unknown effects on transcriptional activity [214]. Human B-cells with CC or GG genotypes show increased *ESR1* mRNA [183]. However, in another study the T allele appeared to be associated with increased *ESR1* enhancer activity, possibly indicating increased transcription [215]. Clinically, the C allele of rs2234693 and the G allele of rs9340799 have been associated with increased serum estradiol in postmenopausal women [216], with no association between these SNPs and serum estrogen or testosterone in older men [216, 217]. Weickert and colleagues observed that in schizophrenia patients, but not controls, the C allele of rs2234693 was associated with decreased *ESR1* mRNA in the frontal cortex [184]. Overall, it appears that variation in these SNPs may alter estrogen signaling via a complex interplay among ER-α expression, ER-α downstream activity, and feedback mechanisms regulating estrogen synthesis. These processes may vary by sex, age, and disease state, making evaluation of the underlying pathways difficult

APOE-ε4 allele may interact with *ESR1* to influence dementia onset [71-73], and our results suggest a possible neural basis for this interaction. rs2234693 significantly interacted with *APOE*-ε4 carrier status in bilateral amygdalae in females, with *APOE*-ε4 positive women in the TT (lowest amygdala volume) group showing lower volumes relative to *APOE*-ε4 negative women. It may be that women carrying a C allele were protected from *APOE*-ε4 -associated deficits in amygdala volume. Biologically, there is evidence that the APOE protein is important in the pathway by which estrogen exerts its neuroprotective effects; these effects are disrupted by the presence of APOE-ε4 protein [178].

Several aspects of this study have notable strengths. Spurious results due to population stratification are unlikely, as only non-Hispanic Caucasian participants were included. The allele frequencies for rs2234693 and rs9340799 were similar to those in other studies [190] and those in the HapMap CEU population in dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP/). Additionally, our imaging methods were completely automated and therefore free from potential investigator-introduced bias. The fact that we showed similar outcomes using VBM and cortical thickness measures add confidence that methodological artifacts do not account for the reported effects. Finally, regression analyses showed that substantial proportions of variance in brain phenotypes (up to 19%) were accounted for by *ESR1* genotype, increasing the likelihood of these effects' potential functional significance.

There are several limitations to this study. First, our sample was comprised of non-Hispanic Caucasian participants, so the results may not generalize to other ethnic and racial groups. Indeed, there is evidence that *ESR1* genotype may differentially influence cognitive decline by ethnicity [70]. Second, females in our study were postmenopausal, and, likely had been for some time. While only one participant was using hormone replacement therapy at the time of the study, the hormonal treatment history of this sample is unknown. With the neural benefits (and likely structural brain effects) of hormonal therapy depending on both timing and formulation of therapy [59, 60], hormonal treatment history would provide valuable information. Treatment history should be considered in future studies designed specifically to test the neural effects of variation in estrogen pathway genes.

To determine how *ESR1* might modulate neurodegeneration, future work will examine the neural correlates of rs2234693 and rs9340799 in MCI and AD populations, Additional studies will be needed to address the effects of *ESR1* in diverse populations, e.g., psychiatric patients and younger people. Functional imaging studies may help address how structural changes might influence clinical

and behavioral phenotypes. Finally, other estrogen-related genes, such as *ESR2* (coding for ER-β) and *CYP19A1* (aromatase) should be studied. Understanding the mechanisms by which alterations in estrogen signaling affect the brain may provide insight into potential etiologies or treatments for the numerous sexually dimorphic psychiatric and neurological disorders.

Summary

In summary, Chapter 1 showed that cognitive complaints and oxidative DNA damage were increased, and memory performance decreased, in BC survivors compared to HC. Imaging results indicated lower gray matter density (GMD) and fMRI activation in several regions in the BC survivor group. PCI was associated with both GMD and activation in the right frontal lobe independent of participant age. GMD in this region was also correlated with global neuropsychological function. In regions where BCS showed decreased GMD this was inversely related to oxidative DNA damage and learning and memory neuropsychological domain scores. This was the first study to show structural and functional effects of PCI, and also the first to relate DNA damage to brain alterations in BCS. The relationship between neuroimaging and cognitive function suggests that these findings have potential clinical relevance. The relationship with peripheral oxidative DNA damage provides a mechanistic clue that warrants further investigation regarding the relationships among cancer- and treatment-related oxidative stress, brain structural changes, and cognition.

The functional brain effects of chemotherapy-induced amenorrhea (CIA) were explored in Chapter 2. We found that combined magnitudes of brain activation and deactivation decreased from pre-to post-chemotherapy in patients undergoing CIA compared to both postmenopausal BC patients undergoing chemotherapy and healthy controls. Furthermore, the change in brain activity magnitude in CIA was strongly correlated with change in processing speed, suggesting this increase in brain activity reflects adaptive cognitive compensation. Our results also demonstrate that the pattern of change in brain activity from pre- to post-chemotherapy varies according to pre-treatment menopausal status. Cognitive correlates add to the potential clinical significance of these findings. These findings have implications for risk appraisal and development of prevention or treatment strategies for cognitive changes in CIA.

In Chapter 3, genetic variation in *ESR1*, the gene coding for estrogen receptor-α, was found to influence hippocampal and amygdalar volumes, particularly in females. The single nucleotide polymorphism rs9340799 influenced cortical gray matter density and cortical thickness differentially by gender. Finally, *APOE*-ε4 carrier status modulated the effect of rs2234693 on amygdala volumes in women; this evidence of epistasis between *APOE* and *ESR1* in influencing brain structure has not been reported previously. This study is the first to demonstrate that, even in cognitively and psychiatrically normal older adults, genetic variation in estrogen signaling widely affects brain morphology in ways that differ by sex, brain region and *APOE*-ε4 carrier status.

Future Directions

To more completely understand the relationship among BC, its treatments, and cognition, several aspects that are missing from the literature should be better studied. To better establish the time course of cognitive and neural changes, patients must be studied prospectively over longer post-treatment intervals—from pre-treatment through years into survivorship. Retrospective studies such as Chapter 1 provide clues, but definitive time course information can only be gleaned from longitudinal studies. The neural effects of tamoxifen and aromatase inhibitors will be crucial factors as well, and should be systematically studied. Currently use of these agents is often treated as a confound due to the focus on chemotherapy and the variability in hormonal treatment regimens. Other cancer treatments, such as antiangiogenic agents, targeted therapies, and immune modulators, are now widely used. The molecular targets of many of these agents are also relevant to brain function, so studying their effectsin future studies will be critical.

Since neuroimaging studies of BC patients must be observational rather than controlled experiments, there are many potential confounds (antidepressant use, smoking status, hypertension, etc.) that affect dependent measures such as BOLD fMRI. Studying a wide range of BC patients and not just a carefully selected group is important for generalizability of results. Sophisticated statistical modeling techniques, such as independent component analysis and nonparametric tests, could be applied to try to differentiate all of these influences. This modeling would be helpful in ascertaining the effects of specific chemotherapy regimens and hormonal treatments and would maximize use of collected data, which is important because these studies are expensive for researchers and burdensome for patients. Choosing the dependent variables, i.e., specific cognitive and neural phenotypes, that are most sensitive to cancer- and treatment-related cognitive dysfunction will be important in this context. Studies such as those in Chapters 1 and 2 contribute to this effort.

Because cancer- and treatment-related changes assessed by neuropsychology and neuroimaging are rather subtle, much larger cohorts than those in the present report will be required to have adequate power to detect and fully characterize these changes. To accomplish this, multicenter collaborations will be critical as it is clear in the current literature that recruitment at a single cancer center cannot provide the hundreds of patients needed for such studies. Recruitment and retention are important in this effort, so utmost care should be taken to maximize efficiency in data gathering and minimize patient burden. This will involve difficult choices regarding which measures to collect. Large cohorts will also allow for meaningful genetic studies which could provide clues to one of the more perplexing (and important) questions in the field: why is only a subset of patients affected by cancer- and treatment-related cognitive dysfunction?

Further study of the biological mechanisms of cancer- and treatment-related cognitive dysfunction is critical for development of neuroprotective, therapeutic, or rehabilitative interventions. Our finding of increased peripheral oxidative damage related to gray matter changes in survivors contributes to this burgeoning field. Systemic inflammation and direct neurotoxicity are thought to damage areas of the brain where neurogenesis occurs, and it is hypothesized that this is part of the pathology underlying cognitive dysfunction [218]. Animal and human studies are needed to explore this hypothesis further. Cognitive dysfunction is part of a constellation of sequelae in cancer patients that includes sleep dysfunction, fatigue, anxiety, and depression, among others. All of these problems are intertwined with the neuroendocrine (i.e., cortisol changes) and immune (including systemic inflammation) disruptions that are known to occur in cancer patients. It will be important for researchers in these disparate fields to collaborate in order to establish a fuller picture of the subjective distress in many areas of life experienced by cancer patients.

Evidence for the efficacy of multiple types of interventions, medical and behavioral, is emerging (see [219] for review). Pharmacologic treatments,

including methylphenidate [220] and fluoxetine [221] show some promise in initial studies. In rodents (but so far not in humans), exercise has been shown to alleviate cognitive dysfunction after chemotherapy [222]. As previously discussed, decreases in estrogen function appear to contribute to cognitive dysfunction in BC. A promising target for drug development is the “neuro-SERM” which would act as an estrogen agonist in brain and bone (to prevent osteoporosis) while being anti-estrogen to breast and uterine tissue to prevent reoccurrence and other malignancy [127, 172]. A “neuro-SERM” is also an attractive option for chemoprevention in women at high risk for BC. All of these interventions are in early stages of study but show promise to help patients in the future.

Further understanding of estrogen’s role in the human brain will contribute to knowledge of BC but is also applicable more generally. Our unexpected finding of an interaction of gender with *ESR1* genotype with regard to cortical gray matter as described in Chapter 3 warrants replication in an independent cohort, which is currently underway. Further, more sophisticated genetic analyses than those employed here will also be helpful: gene-based or pathway analysis, for example, would allow study of genetic variation at multiple points in the estrogen synthetic, response, and degradation pathways using the same number of subjects. This approach utilizes more of the rich genetic dataset available from a genome-wide assay. Pathway analysis also lacks the limitation of dividing participants into discrete genetic groups, which can be problematic given that many influential SNPs have low minor allele frequencies.

Conclusions

In conclusion, this document explored the interplay of breast cancer, estrogen, and cognition using human neuroimaging and genetic methods. The results showed that post-chemotherapy interval, chemotherapy-induced amenorrhea, and genetic variation in an estrogen receptor all influence brain phenotypes. Cognitive correlates of brain findings add to the potential clinical significance of the results. This work provides novel insights into the neural effects of breast cancer treatment. It is hoped that these results inform future research into neuroprotective or therapeutic interventions to preserve brain function in cancer patients as well as those who experience decreases in systemic estrogen.

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Curriculum Vitae

Susan Kim Conroy

Education

2008-2013 PhD, Medical Neuroscience
Indiana University-Purdue University, Indianapolis, IN

2006-2014 Medical Scientist Training Program (MD/PhD)
Indiana University School of Medicine, Indianapolis, IN

2000-2004 BS, Biochemistry with High Distinction
BS, Psychology with High Distinction and Departmental Honors
Indiana University, Bloomington, IN

Research Experience

2008-2012 Doctoral Research
Center for Neuroimaging, Department of Radiology and Imaging Sciences, Indiana University School of Medicine
Research Advisor: Dr. Andrew J. Saykin
Dissertation title: “Breast cancer, estrogen and memory: neuroimaging and genetic variation”
Dissertation committee: Drs. R. Andrew Chambers, Brenna C. McDonald, Kathy D. Miller, and Karmen K. Yoder

2004-2006 Full-time research technician and lab manager
Institute of Psychiatric Research, Department of Psychiatry, Indiana University School of Medicine
Lab director: Dr. R. Andrew Chambers
Animal modeling of dual diagnosis (addiction co-occurring with another psychiatric disorder) using behavioral measures and brain

tissue correlates; neural network computer modeling of learning and neurogenesis

2001-2004 Undergraduate Research, Indiana University Science, Technology, and Research Scholars (IU STARS) program, Indiana University Bloomington
Department of Psychological and Brain Sciences
Research Advisor: Dr. George V. Rebec
Honors Thesis: “Striatal electrophysisology and ascorbate treatment in Huntington Disease transgenic mice”

Awards/Honors

National Institutes of Health, National Institute on Aging individual pre-doctoral MD/PhD fellowship: “Estrogen and memory: genetic variation and neuroimaging” (F30AG039959), 2011-2014

Honorable Mention, IU Simon Cancer Center Research Day, graduate student poster in Clinical/Translational research, 2012

University of Kentucky Chemo Brain Symposium travel award (based on poster judging), 2011

Larry Kays, MD Fellowship (merit-based to top IU Medical Neuroscience graduate students), 2011

Training in Research for Behavioral Oncology and Cancer Control Program Fellow (R25CA117865, PI: V.L. Champion), 2008-2011

University Fellowship, Indiana University-Purdue University Indianapolis, 2006-2007

Undergraduate Excellence in Research Award, Indiana University Bloomington Department of Psychological and Brain Sciences, 2004

Margaret Russell Edmonson Undergraduate Research Award in Genetics, Sigma Xi Scientific Research Society, Indiana University Bloomington, 2004

B.S. awarded with high distinction and departmental honors, Indiana University Bloomington, 2004

Phi Beta Kappa, 2003

Verling and Elizabeth Votaw Scholarship for Summer Undergraduate Research, Indiana University Bloomington Chemistry Department, 2003

Indiana University Bloomington Honors College Grants: Summer Research, 2002, and Senior Thesis, 2004

Howard Hughes Medical Institute Capstone Undergraduate Research Grants: Fall 2002, Spring 2003, and Spring 2004

Indiana University Science Technology and Research Scholars (IU-STARS) undergraduate research program, 2001-2004

Publications

Peer-Reviewed Research Papers

S.K. Conroy, B.C. McDonald, T.A. Ahles, J.D. West, A.J. Saykin (2013). Chemotherapy-induced amenorrhea: a prospective study of brain activation changes and neurocognitive correlates. *Brain Imaging and Behavior* epub ahead of print June 21.

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R.A. Chambers, T.J. Sajdyk, S.K. Conroy, J.E. Lafuze, S.D. Fitz, A. Shekhar (2007). Neonatal amygdala lesions: co-occuring impact on social/fear-related behavior and cocaine sensitization in adult rats. *Behavioral Neuroscience* 121(6):1316-27.

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R.A. Chambers, S.K. Conroy (2007). Network modeling of adult neurogenesis: shifting rates of neuronal turnover optimally gears network learning according to novelty gradient. *Journal of Cognitive Neuroscience* 19(1):1-12 *(also cover art for this issue)*

G.V. Rebec, S.K. Conroy, S.J. Barton (2006). Hyperactive striatal neurons in symptomatic Huntington Disease R6/2 mice: variations with behavioral state and repeated ascorbate treatment. *Neuroscience* 137:327-336.

Book Chapter

S.K. Conroy, B.C. McDonald, D. O'Neill, A.J. Saykin (in press). Utilization and Role of Neuroimaging in Cancer and Oncology. In C.A. Noggle and R.S. Dean, (Eds.), *Neuropsychology of Cancer & Oncology:* Springer

Selected Abstracts

S.K. Conroy, B.C. McDonald, T.A. Ahles, J.D. West, A.J. Saykin (2012). Chemotherapy-induced ovarian failure: brain activation changes and neurocognitive correlates. Presented at IU Simon Cancer Center Research Day May 2012.

K.N. Holohan, Y. Wang, B.C. McDonald, S.K. Conroy, D.J. Smith, J.D. West, A.J. Saykin (2012). Increased cerebral blood flow one month after systemic chemotherapy for breast cancer: a prospective MRI study using pulsed arterial spin labeled perfusion. Presented at IU Simon Cancer Center Research Day May 2012.

S.K. Conroy, B.C. McDonald, D.J. Smith, L.R. Moser, J.D. West, L.M. Kamendulis, J.E. Klaunig, S.M. Perkins, V.L. Champion, F.W. Unverzagt, A.J. Saykin (2012). Brain structure and function in breast cancer survivors: relation to cognitive complaints, oxidative DNA damage and time since chemotherapy. Presented at the International Cognition and Cancer Task Force Meeting, Paris, France March 2012.

B.C. McDonald, S.K. Conroy, D.J. Smith, J.D. West, A.J. Saykin (2012). Breast cancer and treatment effects on brain structure and function in a prospective longitudinal cohort: a replication and extension study. Presented at the International Cognition and Cancer Task Force Meeting, Paris, France March 2012.

K.N. Holohan, S.L. Risacher, S. Swaminathan, J.D. West, M. Inlow, S.K. Conroy, V. Ramanan, T. Foroud, L. Shen, A.J. Saykin, and the Alzheimer's Disease Neuroimaging Initiative (2012). Neural Cell Adhesion Gene Variation and Brain Morphometry in Alzheimer's Disease. Submitted to American Society of Human Genetics 2012.

S.K. Conroy, B.C. McDonald, D.J. Smith, L.R. Moser, J.D. West, S.M. Perkins, V.L. Champion, F.W. Unverzagt, A.J. Saykin (2011). Alterations in brain structure and function after chemotherapy: effect of time since breast cancer treatment. Presented at University of Kentucky Chemobrain Symposium, Lexington KY October 2011.

S.K. Conroy, K. Nho, B.C. McDonald, S.L. Risacher, S. Kim, T. Foroud, C. Jack, Jr., M. Weiner, L. Shen, A.J. Saykin (2011). *ESR1* genetic variation differentially influences brain morphology by sex. Presented at Organization for Human Brain Mapping 17th Annual Meeting Quebec City, Quebec June 2011.

S.K. Conroy, B.C. McDonald, T.A. Ahles, D.J. Smith, J.D. West, F.W. Unverzagt, V.L. Champion, A.J. Saykin (2010). Functional brain abnormalities associated with hormonal therapies for breast cancer in two independent cohorts. Selected for 15-minute platform presentation at International Cognition and Cancer Task Force Meeting, New York, NY March 2010.

A.J. Saykin, B.C. McDonald, T.A. Ahles, J.D. West, S.K.Conroy, L.A. Chesnut, S.A. Horrigan, K.L. Kruck, K. Arfanakis (2008). Chemotherapy-associated changes in brain structure on voxel-based morphometry and diffusion tensor MRI: Preliminary results from a prospective study of breast cancer patients. Presented at 36th Annual International Neuropsychological Society Meetings, February 2008.

R.A. Chambers, T.J. Sajdyk, S.K. Conroy, S.D. Fitz, J. Lafuze, A. Shekhar (2006). Psychiatric and drug response phenotypes in rats with neonatal amygdala lesions. *Neuropsychopharmacology* 31:S1. Presented at American College of Neuropsychopharmacology Meeting, December 2006.

T.J. Sajdyk, S.K. Conroy, S.D. Fitz, J.E. Lafuze, A. Shekhar, R.A. Chambers (2005). Psychiatric and cocaine response phenotypes in rats with neonatal amygdala lesion. Presented at National Institute on Drug Abuse (NIDA) "Frontiers in Addiction Research" Mini-symposium, Nov. 2005.

S.K. Conroy, Z.A. Rodd, R. Chambers (2005). Ethanol sensitization in the neonatal ventral hippocampal lesion rat model of schizophrenia. Program No. 556.6. *2005 Abstract Viewer/Itinerary Planner*. Washington, DC: Society for Neuroscience, 2005. Online.

R.A. Chambers, S.K. Conroy (2005). Variable control of the extent of apoptosis/neurogenesis within mutlilayer plastic networks optimizes learning of new information that varies in degree of novelty. Program No. 143.19. *2005*

Abstract Viewer/Itinerary Planner. Washington, DC: Society for Neuroscience, 2005. Online.

T.J. Sajdyk, S.D. Fitz, C.C. Merrill, S.K. Conroy, R.A. Chambers, A. Shekhar (2005). Behavioral profile of rats following CRF-mediated stressors. Program No. 420.3. *2005 Abstract Viewer/Itinerary Planner*. Washington, DC: Society for Neuroscience, 2005. Online.

T.J. Sajdyk, S.D. Fitz, C. Merrill, S. Conroy, R. Chambers, and A. Shekhar (2005). Behavioral profile of rats following CRF-mediated stressors. *International Behavioral Nerusocience Soc. Abstr 9.*

R.A. Chambers, S.K. Conroy (2005). Variable control of the extent of apoptosis/neurogenesis within multilayer plastic networks optimizes learning of new information that varies in degree of novelty. *Biological Psychiatry* 57(8S): 183S (abstract no. 666)

S.K. Conroy, R.A. Chambers (2005). Ethanol Sensitization in the Neonatal Ventral Hippocampal Lesion Rat Model of Schizophrenia. *Biological Psychiatry* 57(8S): 185S (abstract no. 672)

G.V. Rebec, S.K. Conroy, S.J. Barton (2003). Unit-firing abnormalities in the striatum of HD mice tested under different behavioral conditions. Program No. 390.15. *2003 Abstract Viewer/Itinerary Planner*. Washington, DC: Society for Neuroscience, 2003. Online.

Invited Presentations

Metastatic Breast Cancer Network National Conference (patient and caregiver advocacy organization): “Cancer and treatment-related cognitive dysfunction,” October 2010

Indiana University Simon Cancer Center Grand Rounds: “Neural effects of breast cancer-related hormonal changes,” June 2010

Indiana University Stark Neurosciences Research Institute seminar series: “Neural effects of breast cancer-related hormonal changes,” May 2010

Service

Indiana University School of Medicine MD/PhD Combined Degree Student Council representative, 2008-present

Indiana University School of Medicine Teacher-Learner Advocacy Committee student representative, 2011-present

Ad-hoc reviewer for *Psychooncology* (2010), *Brain Imaging and Behavior* (2011), and *Clinical Breast Cancer* (2011)