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Altered resting brain connectivity in persistent cancer related fatigue

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ABSTRACT

There is an estimated 3 million women in the US living as breast cancer survivors and persistent cancer related fatigue (PCRF) disrupts the lives of an estimated 30% of these women. PCRF is associated with decreased quality of life, decreased sleep quality, impaired cognition and depression. The mechanisms of cancer related fatigue are not well understood; however, preliminary findings indicate dysfunctional activity in the brain as a potential factor. Here we investigate the relationship between PCRF on intrinsic resting state connectivity in this population. Twenty-three age matched breast cancer survivors (15 fatigued and 8 non-fatigued) who completed all cancer-related treatments at least 12 weeks prior to the study, were recruited to undergo functional connectivity magnetic resonance imaging (fcMRI). Intrinsic resting state networks were examined with both seed based and independent component analysis methods. Comparisons of brain connectivity patterns between groups as well as correlations with self-reported fatigue symptoms were performed. Fatigued patients displayed greater left inferior parietal lobule to superior frontal gyrus connectivity as compared to non-fatigued patients ($P < 0.05$ FDR corrected). This enhanced connectivity was associated with increased physical fatigue ($P = 0.04$, $r = 0.52$) and poor sleep quality ($P = 0.04$, $r = 0.52$) in the fatigued group. In contrast greater connectivity in the non-fatigued group was found between the right precuneus to the periaqueductal gray as well as the left IPL to subgenual cortex ($P < 0.05$ FDR corrected). Mental fatigue scores were associated with greater default mode network (DMN) connectivity to the superior frontal gyrus ($P = 0.05$ FDR corrected) among fatigued subjects ($r = 0.82$) and less connectivity in the non-fatigued group ($r = -0.88$). These findings indicate that there is enhanced intrinsic DMN connectivity to the frontal gyrus in breast cancer survivors with persistent fatigue. As the DMN is a network involved in self-referential thinking we speculate that enhanced connectivity between the DMN and the frontal gyrus may be related to mental fatigue and poor sleep quality. In contrast, enhanced connectivity between the DMN and regions in the subgenual cingulate and brainstem may serve a protective function in the non-fatigued group.

1. Introduction

An estimated 3 million women in the United States are living as breast cancer (BC) survivors (American Cancer Society, 2012). While breast cancer is the most widespread type of cancer in women, more patients are in remission mainly because of early detection and important advances in treatment (American Cancer Society, 2014). However, in cancer survivors symptoms of fatigue, pain, poor sleep, and depression are a common occurrence. Persistent cancer related fatigue (PCRF) is one of the most troubling long-term side-effects of cancer treatment (Kim, Son et al., 2008; Alexander et al., 2009; Pearce et al., 2009) and continues to affect around 33% of BC survivors, persisting in some cases for years after completing cancer treatment. The mechanisms of PCRF are largely unknown. Since PCRF is associated with impaired cognition (Rodriguez et al., 2008), decreased sleep quality (Alexander et al., 2009), and depression (Bower, 2005; Kim, Son et al., 2008), it is possible that PCRF has a central neurobiological pathology. In support of this hypothesis, differences in brain metabolites between fatigued and non-fatigued survivors been observed (Zick et al., 2014). It is unknown if a more widespread brain network disturbance may underlie fatigue in this population.

Recent advances in neuroimaging methods have emerged that allow researchers to probe brain network activity and to study altered neural networks non-invasively. One such technique is resting state functional

Abbreviations: PCRF, persistent cancer related fatigue; BCS, breast cancer survivors; fcMRI, functional connectivity magnetic resonance imaging; BOLD, blood-oxygen level dependence; ICA, independent component analysis; MFI, multidimensional fatigue Inventory; BFI, brief fatigue inventory; PSQI, Pittsburgh sleep quality index; HADS, hospital anxiety and depression scale; CFS, chronic fatigue syndrome; MNI, Montreal Neurological Institute; SPM, statistical parametric mapping; FWHM, Full width at half maximum; STR, spatio-temporal regression; FDR, false discovery rate; BA, Brodmann area; DMN, default mode network; SFG, superior frontal gyrus; IPL, inferior parietal lobule; PC, posterior cingulate.

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connectivity magnetic resonance imaging (fcMRI). Using this technique, aberrant brain connectivity patterns have been found in pain (Napadow et al., 2010), depression and insomnia (Li, Wang et al., 2014), two common symptoms also seen in fatigued cancer survivors. However no studies have investigated the role of fatigue in altered brain connectivity in this population. Previous fMRI studies on chronic fatigue syndrome (CFS) have shown changes in brain activations in the superior frontal cortex, premotor, and default mode network (DMN; see below), while performing fatiguing cognitive tasks (Lange et al., 2005; Caseras et al., 2006; Cook et al., 2007). Another study looking at structural changes also showed bilateral decrease in gray matter volume in the prefrontal area among CFS patients as a region that regulates sensations of fatigue (Okada et al., 2004). To explore these regions (and networks) more thoroughly we used resting fcMRI in breast cancer survivors.

There are two fundamental approaches to studying resting state connectivity: seed based approach and a more data-driven method called independent component analysis (ICA). With both approaches functional connectivity is inferred on the basis of correlation between brain regions for time series data from the blood-oxygen level dependence (BOLD) signal. (i) With seed based approaches, a specific region of the brain is chosen a priori based on previous work, and the time course of the BOLD signal in the seed is correlated with each voxel time course of the rest of the brain. Significant correlations are thought to arise when brain regions are "connected". (ii) Data driven multivariate approaches such as ICA do not require a specific prior hypothesis and instead use data from all regions of the brain to identify independent components that function as networks that are connected to other brain regions. With ICA separate resting state networks that show correlated brain activity over time can be assessed for connectivity to other brain regions. Since previous MRI studies have investigated brain outcomes in CFS, we chose to examine similar brain regions using seeds from these studies. In addition we also used ICA to identify novel connectivity patterns as they are not influenced by a prior hypothesis.

One common resting state network is the default mode network (DMN). This network is composed of the medial prefrontal cortex, the posterior cingulate cortex, inferior parietal lobule, and the precuneus (Buckner and Vincent, 2007; Fox and Raichle, 2007). Research over the past decade has shown that this network is activated when a person is at rest, having self-referential thoughts about themselves without engaging with their environment. Recent studies show increased connectivity to this network among chronic pain (Napadow et al., 2010) and depression (Greicius et al., 2007) patients; two symptoms often found in cancer survivors with fatigue. As such, we hypothesized that differences in brain connectivity patterns to the DMN, as well as other brain regions, in fatigued breast cancer survivors may be specifically associated with the subjective symptom of fatigue, and that these differences may be independent of other comorbid symptoms. Here we report an exploratory study which is the first to our knowledge that investigates differences in intrinsic brain connectivity patterns among breast cancer survivors with and without fatigue.

2. Methods

2.1. Participants

The study was approved by the University of Michigan Medical School Institutional Review Board and participants provided written informed consent. Study participants were identified through the University of Michigan Breast Cancer Clinics and from participants in former clinical trials conducted in breast cancer survivors. Eligible participants were women eighteen or older, who have a diagnosis of breast cancer (stage 0 to IIIA), have completed all cancer-related treatments (i.e., surgery, chemotherapy, radiotherapy, immunotherapy, etc.), except hormonal therapy at least 12 weeks prior to the study. Participants were excluded if they: had cancer recurrence; were pregnant or lactating; were diagnosed with anemia with hemoglobin levels less than 12 g/dl or receiving treatment for anemia; were diagnosed with an unstable or untreated comorbidities likely to cause fatigue (i.e., moderate to severe heart failure, hypothyroidism); had a diagnosis of untreated DSM-IV-TR Axis-I or Axis-II disorders; had an initiation, a cessation or change of treatment dose (up to 3 weeks prior to the study start) of any chronic medications or dietary supplements; or if they had metal implants (such as surgical clips or staples) or other contraindications with magnetic resonance imaging (MRI).

During participant screening socio-demographics, height and weight (used to calculated BMI); concomitant medications and supplements; medical history; brief physical including vitals; blood draw for a complete blood count; and a urine pregnancy test were conducted. Menopausal status at time of breast cancer diagnosis was determined through women's medical chart where women who had experienced at least 12 continuous months without a menstrual cycle were deemed post-menopausal. Participants were asked to fill out a battery of self-administered questionnaires such as the multidimensional fatigue Inventory (MFI) (Smets et al., 1995), brief fatigue inventory (BFI) (Mendoza et al., 1999), hospital anxiety and depression scale (HADS) (Zigmond and Snaith, 1983), Pittsburgh sleep quality index (PSQI) (Buysse et al., 1989), and the brief pain inventory (BPI) (Cleeland and Ryan, 1994). For 2 weeks after their initial screening visit, participants were contacted via phone once per week and their BFI score was determined over the phone. To be designated as fatigued BC survivor women needed to have an average BFI $\geq$ 4.0 based on the three BFI administered approximately 1 week apart from their screening visit and via phone contacts on the following 2 weeks. Non-fatigued BC survivors needed an average BFI < 4.0 administered on the same timeframe as fatigued survivors; as well as an average pain score < 4 on BPI, a PSQI total score < 7 and a HADS < 11 for anxiety and depression sub-scales. Non-fatigued patients with significant presentation of these symptoms were excluded as high levels of pain, depression, or sleep problems could influence brain connectivity in that group. These symptoms were not excluded from the fatigue group as cancer survivors with fatigue often have comorbid symptoms of pain, sleep disorders, depression and anxiety, thus making enrollment of purely fatigued patients problematic. To test if our brain connectivity patterns within the fatigued group were related to levels of pain, depression, or sleep problems, we performed bivariate correlations between each brain imaging outcome and comorbid symptom levels within the fatigued group.

2.2. Data acquisition

Participants were recruited to undergo resting state fcMRI on a 3 T Philips Achieva scanner (Best, Netherlands) using an 8 channel head coil. Ten minutes of resting state fMRI data were acquired using a custom T2* weighted spiral-in sequence (repetition time (TR) = 2000 ms, echo time (TE) = 30 ms, flip angle (FA) = 90°, matrix size 80 × 80 with 30 slices, field of view (FOV) = 217 cm, 2.75 × 2.75 × 4 mm voxels and 300 volumes) followed by a T1 weighted high resolution MPRAGE structural scan for normalization using the following parameters [TR = 9.78 ms, TE = 4.59 ms, FA = 90°, FOV = 219 mm, matrix size 240 × 240 matrix with 150 slices and 0.83 × 0.83 × 1 mm voxels]. During the resting state fMRI subjects were instructed not to focus on any particular task and stay awake with their eyes open at a fixation cross. Since cardiac and respiratory fluctuations are known to influence brain connectivity within several networks (Murphy et al., 2013), subject physiological data were collected simultaneously using a chest plethysmograph for respiratory and infrared pulse oximeter on subjects' finger for cardiac data. Only subject functional data of less than 2 mm of translation and less than 1° rotation head motion inside the scanner were included for the fcMRI analysis. Whole brain coverage was achieved including the midbrain and rostral brainstem.

2.3. Data analysis

fMRI data were preprocessed and analyzed using statistical parametric mapping (SPM) software package version 8 (Wellcome Department of Cognitive Neurology, London, United Kingdom), *Conn* (Cognitive and Affective Neuroscience Laboratory, Massachusetts Institute of Technology, Cambridge, USA) functional connectivity toolbox, and GIFT (Group ICA of fMRI Toolbox) toolbar running on MATLAB 7.10 (Mathworks, Sherborn, MA, USA). Upon collection of resting state fMRI data, physiological artifacts were removed using custom Matlab algorithm and slice time corrected using FSL 4.1.9 (FMRIB's Software Library, http://www.fmrib.ox.ac.uk/fsl) software. Preprocessing steps included motion correction, realignment, registration, normalization to standard MNI (Montreal Neurological Institute) template, and smoothing (FWHM Gaussian kernel of 8 mm) using SPM8.

2.3.1. Seed connectivity analysis

Seed to whole brain functional connectivity analysis was done using the *Conn* toolbox (Whitfield-Gabrieli and Nieto-Castanon, 2012). Seed regions were identified from previously published fMRI studies on chronic fatigue syndrome (Lange et al., 2005; Caseras et al., 2006; Cook et al., 2007; Caseras et al., 2008) and created as spheres (5 mm radius) around peak voxel coordinates (Supplementary Table S1). White matter, CSF, and motion parameters were entered into the analysis as covariates of no interest. A band pass filter (frequency window: 0.01–0.1 Hz) was applied to remove linear drifts and high frequency noise from the data. First level analysis was done correlating time course from the seed to whole brain voxels creating connectivity maps for each seed region, using bivariate correlations. These connectivity maps were then passed up to group-level analyses comparing differences in connectivity among fatigued versus non-fatigued BC survivors using age as a covariate of no interest. The resulting maps were threshold at whole brain $P < 0.001$ (or $P < 0.0001$) uncorrected voxel threshold and $P \leq 0.05$ FDR cluster corrected for multiple comparisons. As multiple seeds were chosen ($n = 8$) for our analysis, we also performed a more stringent Bonferroni correction for our results. This threshold was set at $P < 0.0063$ (i.e., 0.05/8 tests). Correlation of brain connectivity outcomes to participant behavioral data were achieved by obtaining the average fisher transformed *r* values of the resulting significant clusters using Marsbar toolbox (Poldrack, 2007), and then correlated with behavioral measures (MFI, BFI and PSQI) in SPSS 21 (Statistical Package for the Social Sciences, IBM Corp., Armonk, NY). Group difference to fatigue measure correlations were done controlling for both pain and depression using linear regression in SPSS. A Bonferroni correction of $P < 0.017$ (i.e., 0.05/3 tests) was also performed on symptom correlations.

2.3.2. Independent component analysis

Group ICA was performed using the GIFT toolbar (Calhoun et al., 2004). Component estimates were validated using ICASSO software (Himberg et al., 2004) for 10 iterations to ensure the reliability of ICA algorithm and to increase the robustness of the results. The number of independent components (ICs) was limited to 25 to minimize splitting into subcomponents. Subject specific spatial maps and time courses were back reconstructed using spatio-temporal regression (STR) or dual regression option available in GIFT. STR regresses (i) the original subject data onto the combined ICA spatial maps to estimate subject specific time courses for each component; and (ii) then regresses the individual subject data back onto these time course matrices to estimate subject specific spatial maps. Thus, the original combined spatial map and the later estimated spatial maps represent the best approximation for the individual subject specific *Z*-score component maps. These *Z* values reflect the degree of connectivity between each voxel and the group averaged time course of the component. Component maps representing resting state networks were identified by spatial correlation with templates provided by Beckmann et al. (2005) and Smith et al. (2009). These individual resting state network maps were then passed onto group second level analyses in SPM where differences in resting state network connectivity between participants with fatigue and non-fatigued participants were performed. We also performed a whole brain covariate of interest interaction analysis using a 2-way ANOVA model with brain connectivity and behavioral measure as factors to assess the differential associations between fatigue symptom levels (MFI and BFI scores) and network connectivity across groups. For all ICA analyses, significant clusters were identified by thresholding resultant brain maps at $P < 0.0001$ uncorrected voxel threshold and $P \leq 0.05$ FDR or FWE cluster corrected significance for multiple comparisons. Since pain and depression are major comorbid symptoms, significant fatigue symptom findings were controlled for both pain and depression in SPM as regressors of no interest.

3. Results

Fifteen women breast cancer survivors with persistent fatigue (average BFI greater than 4) were age-matched (age 57 ± 8 years) to 8 breast cancer survivors without fatigue (age 55 ± 8 years). All participants had fcMRI data that qualified for fMRI analyses. As expected there was no significant differences in age between groups ($P = 0.62$).

3.1. Sociodemographic and clinical characteristics:

The sociodemographic and clinical characteristics by fatigue status are presented in Table 1. Fatigued women had significantly greater BFI

Table 1
Sociodemographic and clinical characteristics by disease status.

Demographics	Non-fatigued ($N = 8$)	Fatigued ($N = 15$)	*P*-value
Age, Mean, SD	55 ± 8.1	57 ± 8.7	0.62[a]
Race, N (%) white	8 (100.0)	15 (100.0)	0.99[b]
Breast cancer stage, N (%)			0.41[b]
0	1 (12.5)	1 (6.7)	
1	3 (37.5)	6 (40)	
2	1 (12.5)	6 (40)	
3	3 (37.5)	2 (13.3)	
Breast cancer treatments, N (%)			
Chemotherapy	7 (87.5)	10 (66.7)	0.28[b]
Radiation	7 (87.5)	11 (73.3)	0.43[b]
Surgery	8 (100.0)	15 (100.0)	0.99[b]
Hormone therapy	3 (37.5)	11 (73.3)	0.09[b]
Time since diagnosis, Mean, SD (months)	82 ± 35.4	77 ± 47.4	0.79[a]
Post-menopausal status, N (%)	4 (50.0)	8 (53.3)	0.88[b]
ER+ receptor status, N (%)	2 (25.0)	12 (80.0)	0.01[b]
BMI, Mean, SD	28.9 ± 4.9	29.5 ± 6.9	0.82[a]
Multidimensional fatigue inventory (MFI)			
General fatigue, Mean, SD	11.75 ± 3.73	15.80 ± 4.29	0.03[a,c]
Physical fatigue, Mean, SD	8.25 ± 4.06	12.0 ± 4.84	0.07[a]
Reduced activation, Mean, SD	9.0 ± 3.78	13.40 ± 5.56	0.05[a,c]
Reduced motivation, Mean, SD	8.38 ± 2.13	9.87 ± 3.56	0.29[a]
Mental fatigue, Mean, SD	8.50 ± 4.95	13.60 ± 3.20	0.007[a,c]
Brief fatigue inventory (BFI)			
BFI — severity, Mean, SD	9.0 ± 5.09	16.8 ± 4.67	0.001[a,c]
BFI — interference, Mean, SD	8.25 ± 6.69	22.53 ± 11.21	0.004[a,c]
PSQI, Mean, SD	6.25 ± 2.96	8.07 ± 3.84	0.25[a]
Hospital anxiety depression scale (HADS)			
Anxiety, Mean, SD	3.75 ± 3.65	7.07 ± 4.96	0.11[a]
Depression, Mean, SD	2.50 ± 2.26	6.53 ± 5.05	0.01[a,c]
Brief pain inventory (BPI), Mean, SD-			
Severity	0.59 ± 1.01	2.83 ± 2.45	0.02[a,c]
Interference	0.45 ± 1.05	2.52 ± 2.57	0.04[a,c]

[a] *P*-value is based on an independent sample *t*-test.
[b] *P*-value is based on a Pearson Chi-square test.
[c] *P*-value is significant at ≤ 0.05.

severity ($P < 0.001$), BFI interference ($P < 0.004$), MFI general fatigue ($P < 0.03$), MFI mental fatigue ($P < 0.007$), HADS depression ($P < 0.01$), BPI severity ($P < 0.02$), BPI interference ($P < 0.04$). There was no significant difference in the type of chemotherapy received between groups ($P = 0.80$), a majority of them ($n = 15$) received a combination of Cytoxan, Adriamycin and Taxol treatment.

3.2. Analysis 1: Seed to whole brain connectivity

Using seeds from previous chronic fatigue patient studies showing differences in brain activations during cognitive tasks (see Supplementary Table S1), we identified multiple regions showing significantly greater connectivity in fatigued BC survivors as compared to non-fatigued participants. These regions included connectivity between the: left inferior parietal lobule (IPL) to right superior frontal gyrus (SFG) ($P = 0.003$ FDR corrected), right medial frontal gyrus/Brodmann area 11 (BA11) to right inferior parietal lobule ($P = 0.003$ FDR corrected), precuneus to anterior insula ($P = 0.05$ FDR corrected) and posterior cingulate to cerebellum ($P = 0.001$ FDR corrected) [see Fig. 1, Table 2]. Of these regions the IPL to SFG, medial frontal gyrus to IPL and posterior cingulate to cerebellum survived a more stringent Bonferroni correction given the number of seeds chosen. We did not see any significant results with any other

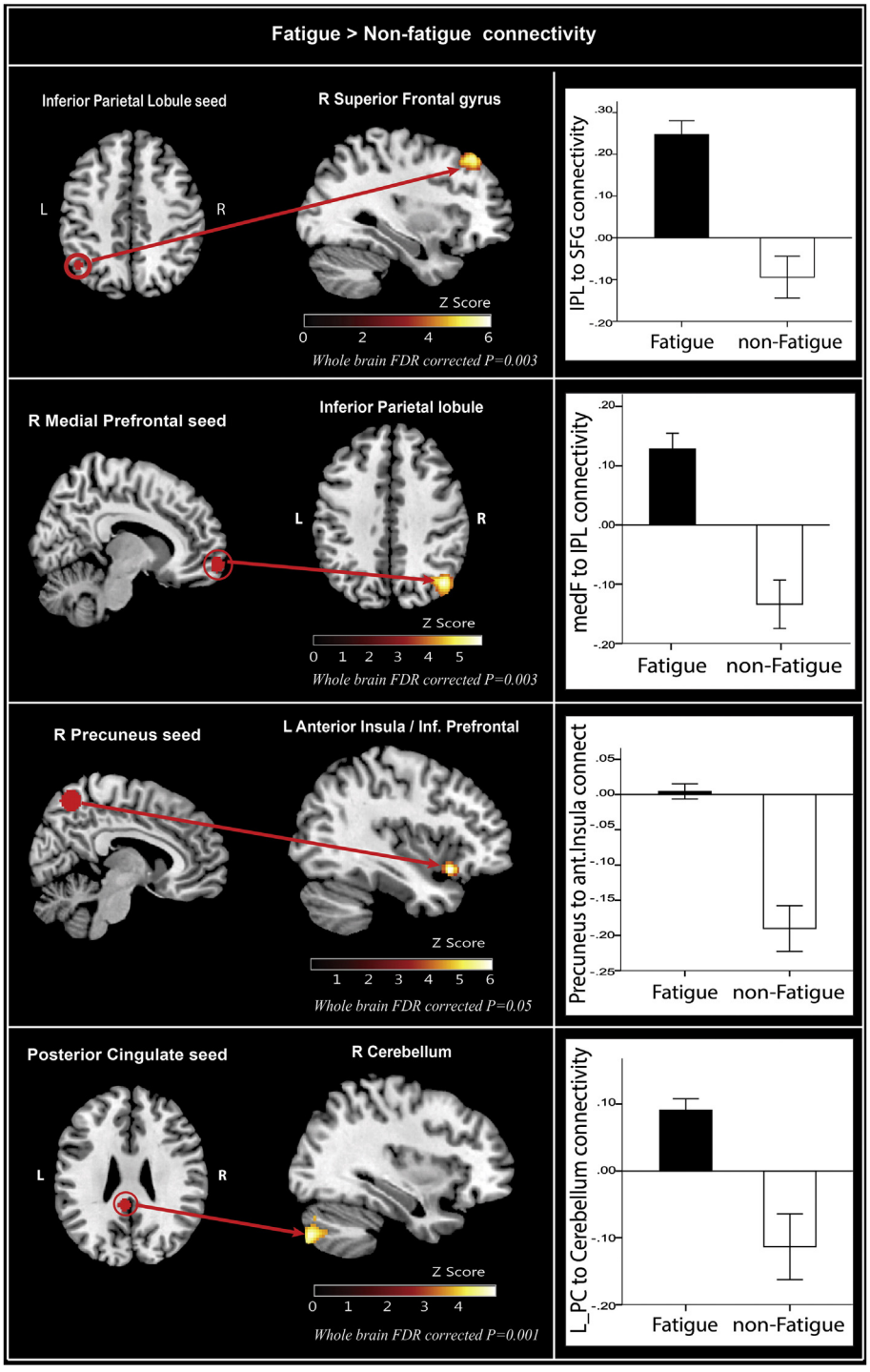


Fig. 1. Greater brain resting state connectivity in fatigued BC survivors compared to non-fatigued survivors. Brain images show greater intrinsic connectivity between seed (left) to other brain regions (middle). Bar graphs (right) show the level of connectivity between seed and connected region in both groups using Fisher transformed *R*-values (*y*-axis).

seeds included from the previously published studies on chronic fatigue syndrome.

In contrast, significantly greater connectivity was seen within the non-fatigued BC survivors between the: precuneus to PAG ($P = 0.003$ FDR corrected), inferior parietal lobule to parahippocampus ($P = 0.02$ FDR corrected), left inferior parietal lobule to subgenual anterior cingulate cortex (sgACC) ($P = 0.03$ FDR corrected), left posterior cingulate to middle temporal gyrus ($P = 0.008$ FDR corrected) and precuneus to primary somatosensory cortex/post central gyrus ($P = 0.05$ FDR corrected) (see Fig. 2). Of these regions the posterior cingulate to middle temporal gyrus connectivity survived Bonferroni correction given the number of seeds chosen. There was no effect of either hormone (all $P > 0.30$) or ER + receptor status (all $P > 0.09$) on significant brain connectivity differences found between groups.

3.3. Behavioral correlations among fatigued survivors

While controlling for co-morbid symptoms, connectivity between the left IPL and SFG, as identified in our seed based analyses, was associated with increased physical fatigue, while controlling for sleep (subscale from MFI questionnaire: $P = 0.001$; $r = 0.76$), and poor sleep while controlling for fatigue (PSQI: $P = 0.002$; $r = 0.72$) in the breast cancer survivors with fatigue (Fig. 3A). Similarly, right medial frontal gyrus to IPL connectivity showed a significant positive correlation to MFI-mental fatigue ($P = 0.03$; $r = 0.54$) in the fatigued survivors (Fig. 3B). Of these correlations the relationship between IPL to SFG and physical fatigue and between IPL to SFG and sleep quality (Fig. 3A) survived Bonferroni correction for multiple comparisons. No other significant correlations were seen between brain connectivity and other clinical symptoms such as Pain, depression, anxiety and sleep ($P > 0.12$).

3.4. Analysis 2: ICA interaction analysis using subjective mental fatigue scores.

Six components were identified as resting state networks according to network maps from Beckmann et al. (2005) default mode network (DMN); dorsal attention network, salience network, right and left frontal control network and sensory motor network. There were no significant differences in component maps between groups. Doing an interaction analysis using mental fatigue scores as a covariate of interest, subjects with persistent fatigue demonstrated a positive correlation between intrinsic DMN connectivity to the superior frontal gyrus (SFG) whereas non-fatigued patients displayed the opposite relationship ($P = 0.05$ FDR cluster corrected; no. of voxels = 151; Z score = 4.77; MNI peak voxel coordinates (x, y, z) = (−35, 31, 37)) (see Fig. 4). This relationship remained significant after correcting for comorbid depression ($P = 0.02$ FWE cluster corrected) and pain (0.04 FWE cluster corrected). No other significant interactions were found between the networks and other clinical symptoms.

4. Discussion

Here we report the first study to link self-reported fatigue to intrinsic brain connectivity outcomes in women with persistent cancer related fatigue. Specifically connectivity between the DMN and regions within the superior frontal gyrus is increased in these individuals as compared to non-fatigued breast cancer survivors. Moreover, the degree of increased connectivity was correlated with self-reported clinical symptoms of fatigue and sleep quality.

Specifically, we observed greater connectivity among fatigued breast cancer survivors between the IPL to superior frontal gyrus (SFG), medial prefrontal to IPL, precuneus to anterior insula, and posterior cingulate (PC) to cerebellum. In the fatigued group, the degree of this increased connectivity between the IPL to SFG was positively correlated to women's physical fatigue scores and poor sleep quality. The medial prefrontal to IPL connectivity also showed a positive correlation to subjective mental fatigue scores. Interestingly, we found similar result from our ICA network analysis: subjective levels of mental fatigue were associated with increased connectivity between the DMN and the superior frontal gyrus in the fatigued patients. These results may reflect an altered DMN response to internal sensory input for this fatigued population.

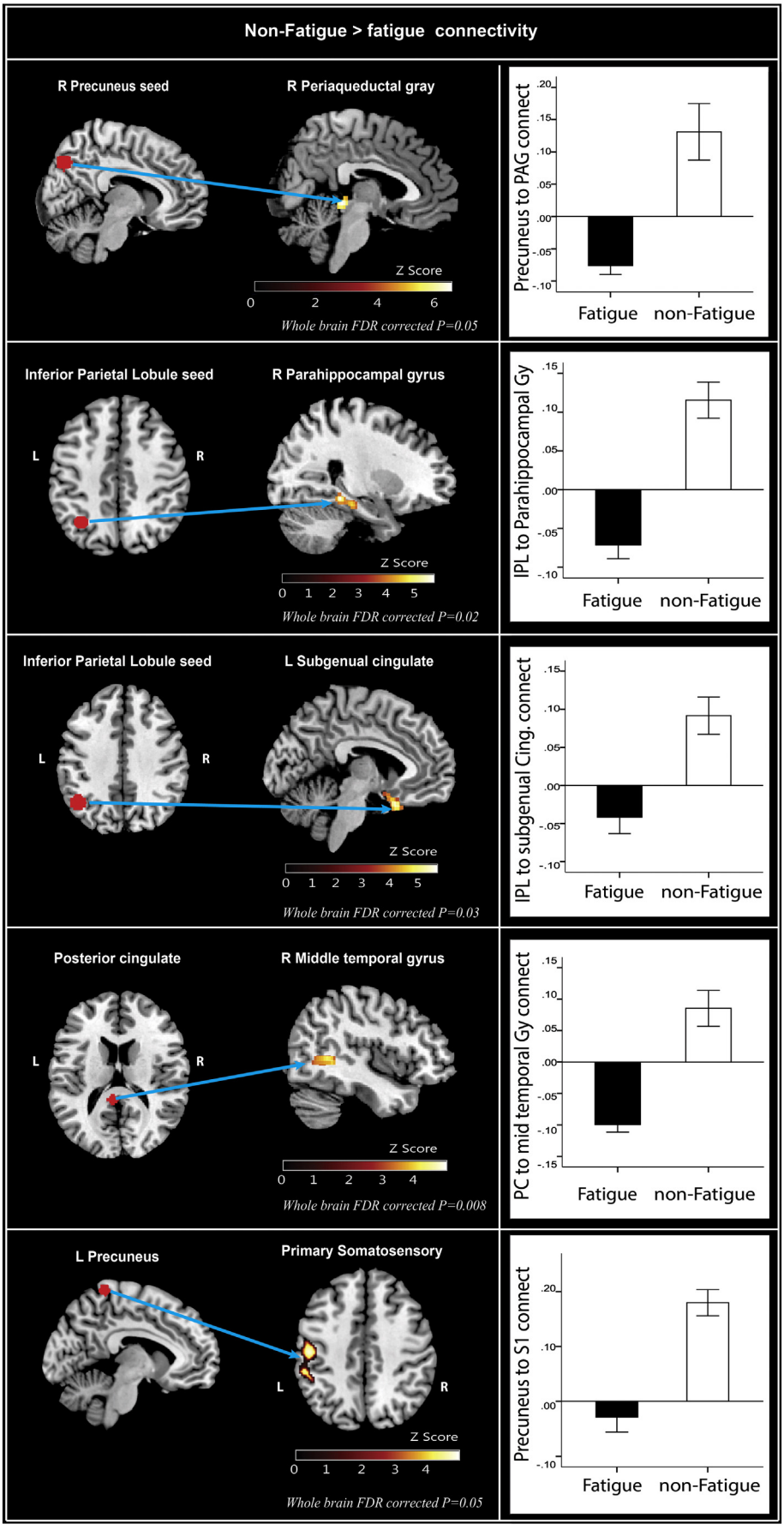
The SFG and the medial prefrontal cortex region are key nodes whose activity is associated with intrinsic connectivity in subjects with PCRF. The SFG is a region shown previously to be involved in disrupted cognition and poor memory among chronic fatigue syndrome population (Lange et al., 2005). Decreases in gray matter volume within this structure was found to parallel poor cognition and increased fatigue severity in chronic fatigue syndrome subjects (Okada et al., 2004). These data implicate that the SFG as playing a role in fatigue and overuse of this region might cause decreases in gray matter due to decrease in the number of inhibitory neurons as a result of maladaptive compensation. Our data also support the notion that abnormalities in SFG connectivity may be related to fatigue in cancer survivors. To investigate the role of this particular region of the SFG in other studies we performed a literature search using the identified SFG coordinates in neurosynth.org (Yarkoni et al., 2011), this resulted in 1047 published studies reporting involvement of this region in memory processing.

The medial prefrontal cortex is part of the DMN network along with posterior cingulate, precuneus and IPL (Buckner and Vincent, 2007; Fox

Table 2
Differences in brain connectivity between fatigued and non-fatigued BC survivors (seed based analysis).

Seed regions	Connectivity region	Brodmann area	Cluster size (# of voxels)	Z-score (peak value)	Peak voxel coordinates (MNI space)		
					X	Y	Z
1. Fatigue > non-fatigue							
Left inferior parietal lobule	Superior frontal gyrus[ab]	BA 8	400	4.35	34	28	48
Right superior/medial frontal gyrus	Inferior parietal lobule[ab]	BA 39	347	4.35	44	−68	34
Right precuneus	Anterior insula[c]	BA13	76	4.78	−28	20	−16
Posterior cingulate	Cerebellum[ab]	NA	461	3.94	52	−72	−40
2. Non-fatigue > fatigue							
Right precuneus	Periaqueductal gray (PAG) [c]	NA	141	6.61	2	−34	−4
Inferior parietal lobule/primary somatosensory	Right parahippocampus[a]	BA 35	252	5.61	26	−36	−12
Left inferior parietal lobule	Subgenual anterior cingulate[a]	BA 25	277	4.47	−4	16	−26
Left posterior cingulate	Middle temporal gyrus[ab]	BA 21	322	4.43	40	−54	4
Left precuneus	Primary somatosensory[a]	BA 4	231	3.72	−48	−18	42

[a] Voxel threshold were set at $P < 0.001$ and $P < 0.05$ cluster FDR correction.
[b] Significant after Bonferroni correction for multiple comparisons.
[c] Voxel threshold were set at $P < 0.0001$ and $P < 0.05$ cluster FDR correction; NA = none applicable.


Non-Fatigue > fatigue connectivity
R Precuneus seed
R Periaqueductal gray
Z Score
0 2 4 6
Whole brain FDR corrected P=0.05
Precuneus to PAG connect
.20 .15 .10 .05 .00 -.05 -.10
Fatigue non-Fatigue
Inferior Parietal Lobule seed
R Parahippocampal gyrus
L R
Z Score
0 1 2 3 4 5
Whole brain FDR corrected P=0.02
IPL to Parahippocampal Gy
.15 .10 .05 .00 -.05 -.10
Fatigue non-Fatigue
Inferior Parietal Lobule seed
L Subgenual cingulate
L R
Z Score
0 1 2 3 4 5
Whole brain FDR corrected P=0.03
IPL to subgenual Cing. connect
.15 .10 .05 .00 -.05 -.10
Fatigue non-Fatigue
Posterior cingulate
R Middle temporal gyrus
L R
Z Score
0 1 2 3 4
Whole brain FDR corrected P=0.008
PC to mid temporal Gy connect
.15 .10 .05 .00 -.05 -.10 -.15
Fatigue non-Fatigue
L Precuneus
Primary Somatosensory
L R
Z Score
0 1 2 3 4
Whole brain FDR corrected P=0.05
Precuneus to S1 connect
.20 .10 .00 -.10
Fatigue non-Fatigue

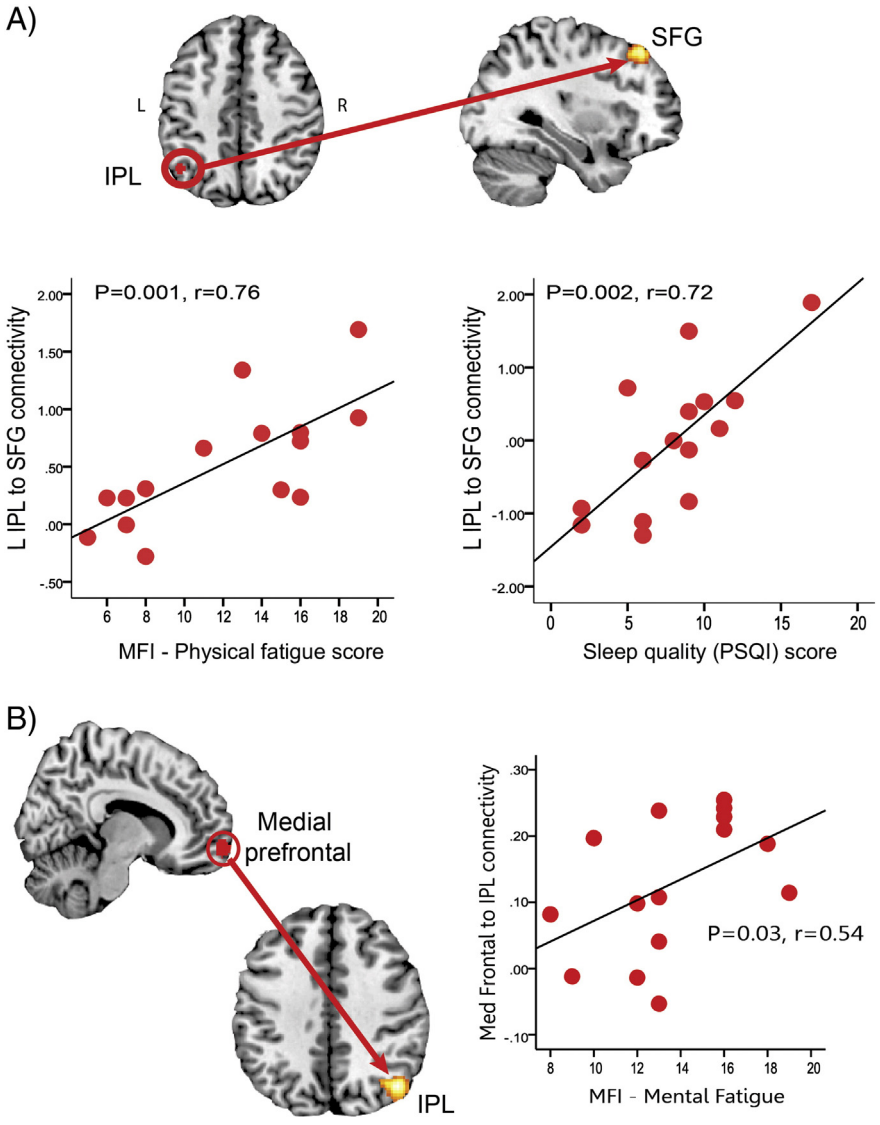


Fig. 3. Increased brain connectivity to the DMN in fatigued BC survivors is associated with clinical symptoms of fatigue and poor sleep. Scatter plots show positive correlations for inter-individual differences in brain connectivity (Fisher transformed *r*-values; *y*-axis) and self-reported fatigue or sleep disturbance (*x*-axis).

and Raichle, 2007). The DMN is a constellation of brain regions involved in self-referential thinking and it is thought to mediate processes that are important for the resting brain (Raichle et al., 2001; Fox et al., 2005). The DMN is typically deactivated when focused on external tasks (Fox and Raichle, 2007). Interestingly, despite the large number of seeds investigated in the seed to whole brain connectivity analysis, only the regions associated with the DMN demonstrated significant differences between fatigued and non-fatigued survivors. We hypothesize that increased intrinsic connectivity to this network may be accompanied by or related to additional mental processes leading to fatigue. Previous findings by our group have also shown increased DMN connectivity to the insula in chronic pain patients diagnosed with fibromyalgia (Napadow et al., 2010): greater insula to DMN connectivity was associated with more clinical pain. Since fatigue is also a common symptom in fibromyalgia, it is interesting to note that while pain and fatigue symptoms are both related to DMN connectivity, the regions that the DMN is connected to appear to have some symptom specificity: namely insula for chronic pain and SFG for fatigue. Interestingly other studies have also shown altered connectivity with the DMN in conditions such as chronic lower back pain, obsessive compulsive disorder, and Alzheimer's disease (Beucke et al., 2014; Zhang, Wu et al., 2014; Zhong, Huang et al., 2014).

We also observed increased connectivity patterns in non-fatigued subjects as compared to the matched fatigued BC survivors. Non-fatigued subjects showed greater brain connectivity between anti-nociceptive regions and the DMN. For example, the precuneus and IPL showed greater connectivity to anti-nociceptive regions such as the periaqueductal gray (PAG) and subgenual anterior cingulate (sgACC). Within the endogenous antinociceptive system the PAG plays a central role along with cingulate and prefrontal cortex in coordinating descending pathway to decrease nociceptive routing (Heinricher et al., 2009; Schweinhardt and Bushnell, 2010). As reported in our earlier study (Zick et al., 2014), we observed greater clinical pain scores (brief pain inventory questionnaire) in our fatigued subjects (Mean ± SD =

Fig. 2. Increased brain resting state connectivity in non-fatigued as compared to fatigued BC survivors. Brain images show significant intrinsic connectivity seen between seed (left) to other brain regions (middle). Bar graphs (right) show level of connectivity in both groups between seed and connected region using Fisher transformed *R*-values (*y*-axis). All results significant at $P < 0.05$ FDR corrected.

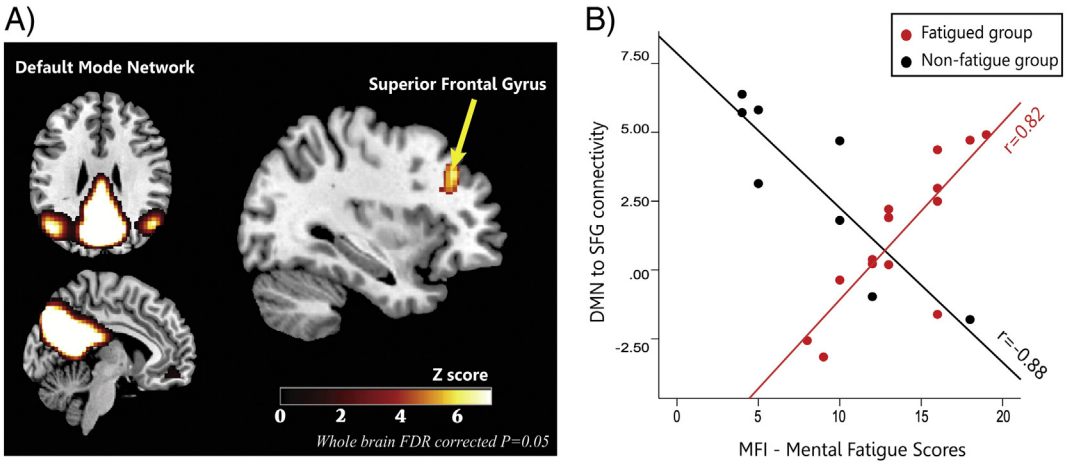


Fig. 4. Differential relationship between self-reported mental fatigue and DMN connectivity to the superior frontal gyrus in BC survivors with and without persistent fatigue. (A) Brain images show altered DMN connectivity to SFG in association to mental fatigue between groups. (B) Scatter plot shows differential relationship for mental fatigue scores between the fatigued group (red; Mean ± SD: 13.6 ± 3.2) and non-fatigued group (black; Mean ± SD: 8.5 ± 4.9) and DMN-SFG connectivity. Increased mental fatigue is associated with increased DMN-SFG connectivity among BC survivors with fatigue. The opposite relationship is seen among the non-fatigued group. *Z*-scores representing the level of DMN to SFG connectivity are plotted on the *y*-axis and subjective mental fatigue scores are plotted on the *x*-axis.

2.83 ± 2.5) as compared to the non-fatigued (Mean ± SD = 0.59 ± 1.1) group. Since individuals pain, fatigue and sleep quality co-vary together in chronic pain (Bennett et al., 2007) and cancer (Roscoe et al., 2007) patient populations, we speculate that this increased connectivity between DMN to anti-nociceptive regions may decrease their perception of pain and as a result lower their fatigue levels. In addition, previous studies have shown increased PAG to DMN connectivity associated with subjects' tendency to mind wander or paying less attention to pain (Kucyi et al., 2013). This raises the possibility that a similar mechanism of perceptual disengagement of attention may result in less attention being paid to fatigue symptoms.

Interestingly both fatigued and non-fatigued BCS showed opposite relationship between mental fatigue report and DMN network connectivity to the SFG. Greater connectivity among the fatigue group was associated with more mental fatigue and less connectivity among the non-fatigued group was associated with less or no mental fatigue. This supports our current understanding in the literature that DMN connectivity is modulated due to patient rumination (Kucyi et al., 2014). Decreased DMN connectivity to SFG among the non-fatigued group could indicate non-fatigued subjects cogitate less on their condition compared to fatigued BC subjects.

We acknowledge that our study has a number of limitations. Our study involved a relatively small sample of participants, thus potentially limiting the generalizability of our findings, also since this study was cross-sectional in design we cannot say whether the increased connectivity to the SFG, for example, is causing or a consequence of fatigue. We also understand that functional brain connectivity does not show a causal relationship for the direction of connectivity. So the enhanced connectivity seen might be a consequence of PCRF instead of being the cause. Furthermore, our participant sample was homogeneous being largely made up of white women, so our findings may not apply to other races. We also were aware that our findings may be related to other comorbid symptoms that these patients display, and as such, we attempted to control for pain and depression in our more robust findings. That said, we recognize that there might be other undiagnosed or unrecognized conditions that we could not control for in our analyses. Finally, since there was no previous fMRI study done on PCRF other than ours (Zick et al., 2014), our seed connectivity results are reported as an exploratory analysis. As such, results that were not significant after Bonferroni correction for multiple comparisons should be interpreted with caution. Finally, our seed connectivity findings were based on previous literature in chronic fatigue syndrome. As such, there may be other brain regions or networks contributing to fatigue symptoms that have yet to be identified.

In conclusion, this is the first study looking at intrinsic brain connectivity in relationship to PCRF. Our findings have implications for underlying brain mechanisms for persistent fatigue among breast cancer survivors. The overlapping SFG region, which was identified using two different analysis techniques, might potentially point toward this region as being a marker for tracking fatigue among BCS. We also speculate that the enhanced connectivity between the DMN and SFG may be related to impaired cognition and poor sleep quality often seen in women with PCRF. However we did not perform any cognitive tasks to assess cognition. Future studies with larger populations are needed, to study cognition, to replicate results and to track changes in the brain outcomes both during development and through time with treatment. As our previous connectivity analyses in chronic pain conditions have been associated with successful treatments (Napadow et al., 2012; Harris et al., 2013), similar findings may help identify possible treatment options for persistent cancer related fatigue.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.nicl.2015.04.022.

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