Abnormal Resting-State Functional Connectivity in Patients with Chronic Fatigue Syndrome: Results of Seed and Data-Driven Analyses

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Abstract

Although altered resting-state functional connectivity (FC) is a characteristic of many chronic pain conditions, it has not yet been evaluated in patients with chronic fatigue. Our objective was to investigate the association between fatigue and altered resting-state FC in myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS). Thirty-six female subjects, 19 ME/CFS and 17 healthy controls, completed a fatigue inventory before undergoing functional magnetic resonance imaging. Two methods, (1) data driven and (2) model based, were used to estimate and compare the intraregional FC between both groups during the resting state (RS). The first approach using independent component analysis was applied to investigate five RS networks: the default mode network, salience network (SN), left frontoparietal networks (LFPN) and right frontoparietal networks, and the sensory motor network (SMN). The second approach used *a priori* selected seed regions demonstrating abnormal regional cerebral blood flow (rCBF) in ME/CFS patients at rest. In ME/CFS patients, *Method-1* identified decreased intrinsic connectivity among regions within the LFPN. Furthermore, the FC of the left anterior midcingulate with the SMN and the connectivity of the left posterior cingulate cortex with the SN were significantly decreased. For *Method-2*, five distinct clusters within the right parahippocampus and occipital lobes, demonstrating significant rCBF reductions in ME/CFS patients, were used as seeds. The parahippocampal seed and three occipital lobe seeds showed altered FC with other brain regions. The degree of abnormal connectivity correlated with the level of self-reported fatigue. Our results confirm altered RS FC in patients with ME/CFS, which was significantly correlated with the severity of their chronic fatigue.

Key words: chronic fatigue; connectivity; fMRI; ICA.

Introduction

MYALGIC ENCEPHALOMYELITIS/CHRONIC fatigue syndrome (ME/CFS) affects over 1.3 million individuals in the United States and reduces the quality of life to levels comparable to people with congestive heart failure (Komaroff et al., 1996). Treatments for ME/CFS remain largely ineffective at reducing illness-related symptoms. To improve the care of these individuals, a greater understanding of the pathophysiology of ME/CFS is needed. Recent work in this area has examined the role of abnormal immunity as a pathway leading to ME/CFS (Hornig et al., 2015), while less attention has focused on the central mechanisms of persistent fatigue.

Fatigue is a common symptom in the general population, but usually resolves with sleep (Akerstedt et al., 2014). ME/CFS, however, is a condition characterized by disabling fatigue and cognitive abnormalities that seem to be unresponsive to rest. Similar but often less severe fatigue has been reported in other chronic conditions, such as depression, arthritis, and chronic pain, where symptoms persist for long periods of time (Katz et al., 2015; Kroenke et al., 2011; Maes et al., 2012).

With advances in neuroimaging, the brain's role in persistent conditions, like ME/CFS, has become an important focus of research. Previous investigations of brain abnormalities in ME/CFS were mostly inconclusive (Lange et al., 1998). There is conflicting evidence for altered brain structure in this illness, with some studies reporting either globally decreased gray matter or local abnormalities in several brain regions (Puri et al., 2012 and Okada et al., 2004), while others report no such findings (Perrin et al., 2010).

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Global or regional reductions in cerebral blood flow (CBF) at rest have been reported in ME/CFS (Biswal et al., 2011; Cope and David, 1996; Tirelli et al., 1998; Yoshiuchi et al., 2006), but some investigators found no group differences in CBF compared to normal controls (Perrin et al., 2010). However, functional MRI (fMRI) has shown altered cortical activation during cognitive challenges in ME/CFS (De Lange et al., 2004; Tanaka et al., 2006).

Resting-state functional connectivity (FC) is often disrupted in chronic conditions, such as fibromyalgia, back pain, and temporomandibular joint disorder, and may contribute to prolonged symptoms (Baliki et al., 2011; Ichesco et al., 2014; Schmidt-Wilcke et al., 2014; Tagliazucchi et al., 2010). However, FC is yet to be investigated in patients with ME/CFS. Examining FC between brain areas in the absence of a task (i.e., resting state [RS]) may offer some insights as to why such patients have considerable difficulty in performing challenging tasks, such as the Psychomotor Vigilance Test (Lim et al., 2010).

Two general approaches can be used to evaluate the FC among brain regions. One requires prior knowledge of seed regions (i.e., model based), while the other is driven by the acquired data (i.e., data driven). The results of these approaches are complementary and, when combined, may provide powerful insights about the neural underpinnings of clinical disorders, like ME/CFS.

In this study, we used both approaches to assess FC in patients with ME/CFS. Our data-driven approach was similar to the one used in patients diagnosed with fibromyalgia (Napadow et al., 2010). We investigated FC among brain regions in ME/CFS patients and normal nonfatigued healthy controls (HC) in five resting-state networks (RSN): the default mode network (DMN), salience network (SN), sensory motor network (SMN), and the left and right frontoparietal networks (LFPN, RFPN). We hypothesized that self-reported fatigue would be associated with altered FC within these networks. Moreover, compared to HC, we expected the RS among ME/CFS patients to connect to additional brain regions such as the insula, parietal, and frontal lobes.

For the second set of analyses, we used the model-based approach to test FC among brain regions, using seed regions that had demonstrated decreased resting regional cerebral blood flow (rCBF) in ME/CFS patients through arterial spin labeling (ASL). Those regions were located in the parahippocampal and occipital lobes. Given the reductions in rCBF within these brain clusters, we expected the FC with other brain regions to be disrupted and associated with reports of fatigue in patients with ME/CFS.

Methods and Procedures

Participants

Individuals with chronic fatigue who fulfilled the 1994 Case Definition (Fukuda et al., 1994) as well as the 2003 Canadian Criteria for ME/CFS were recruited from outpatient clinics at the University of Florida. Only female subjects were enrolled because of the preponderance of women in ME/CFS. Following the recruitment of clinical subjects, nonfatigued healthy individuals were invited to participate through local advertising to serve as age- and sex-matched controls. Subjects with a history of heart disease, COPD, malignancy, or other systemic disorders were excluded, including those with psychiatric

illnesses that would be exclusionary for a diagnosis of ME/CFS (Reeves et al., 2003). The work described in this article was carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. All participants provided informed consent before participation. The University of Florida Institutional Review Board approved all study procedures before the start of study enrollment.

Multidimensional fatigue inventory

The multidimensional fatigue inventory (MFI) is a self-reported instrument that contains 20 statements covering different aspects of fatigue (Smets et al., 1995). The 20 items are organized into 5 scales: general fatigue, physical fatigue, reduced activity, reduced motivation, and mental fatigue. Higher scores on the questionnaire indicate more fatigue.

Neuroimaging

Acquisition. All neuroimaging data were acquired with a research-dedicated Philips Achieva 3T whole body MRI scanner using a 32-channel head coil. Three types of data were obtained during a single scanning session: (A) A high-resolution 3D anatomic structural scan; (B) a resting-state ASL scan; and (C) a blood oxygen level-dependent (BOLD) resting-state scan. The parameters for the anatomical T_1-weighted MPRAGE structural scan were as follows: 176 slices in sagittal orientation, $TR = 7$ msec, $TE = 3.2$ msec, acquisition matrix $= 240$ mm $\times 240$ mm, and voxel size $= 1$ mm^3. The ASL sequence used a pseudocontinuous ASL design with the following parameters: 20 slices in transverse orientation, slice gap $= 1$ mm, $TR = 4000$ msec, $TE = 11$ msec, label duration $= 1500$ msec, postlabel delay $= 1800$ msec, acquisition matrix $= 72$ mm $\times 72$ mm, voxel size $= 3.19$ mm $\times 3.19$ mm $\times 6$ mm, and number of dynamic scans (pairs) $= 45$. Finally, the resting-state BOLD scans were acquired using an echo planar acquisition protocol with the following parameters: 42 slices in transverse orientation, slice gap $= 0$ mm, $TR = 2250$ msec, $TE = 30$ msec, acquisition matrix $= 80$ mm $\times 80$ mm, and 3 mm isotropic voxels. The RS ASL scans were obtained to identify brain areas demonstrating rCBF differences between ME/CFS subjects and NC to be subsequently used as seeds in our model-based approach.

Resting-state CBF. Statistical Parametric Mapping 8 (SPM8) (Wellcome Department of Imaging Neuroscience, London, United Kingdom; http://www.fil.ion.ucl.ac.uk/spm) software was used for ASL data preprocessing. Label and control ASL images were independently processed through SPM8's realign motion correction algorithm, and a combined mean realigned image was created. The raw ASL images were then resliced with the motion correction parameters generated by the previously described step. The realigned resliced images were then smoothed with a 6 mm full-width half-maximum (FWHM) Gaussian kernel. Using the MATLAB toolbox ASLtbx (Wang et al., 2008), subtractions between labeled and controlled images were performed resulting in 45 perfusion images (one for each dynamic scan). CBF values were then calculated on a per voxel basis for each perfusion image and then averaged (over the 45 images) to create one mean CBF image. For a detailed description of the CBF calculation, see Wang et al., 2008.

The mean CBF image was then normalized to the Montreal Neurologic Institute (MNI) standardized space using the parameters generated by SPM's segmentation normalization procedure and resliced to $2\,mm^3$ voxels. Finally, the normalized mean CBF image was masked with SPM's default brain mask with a 50% threshold to exclude outlier voxels. This process was repeated for each participant.

SPM12 was used to perform independent sample t-tests to compare CBF between the ME/CFS and HC groups. Contrasts were created to identify cortical regions where CBF was increased or decreased in clinical subjects compared to controls. These contrasts produce maps with a t-statistic for every voxel in the brain. The resulting t maps were thresholded at a t-statistic of $p < 0.001$ and a cluster size of $120\,mm^3$ to control for the large number of multiple comparisons associated with voxelwise analyses.

BOLD data preprocessing. Functional imaging data were preprocessed in SPM12 (Wellcome Department of Imaging Neuroscience; http://www.fil.ion.ucl.ac.uk/spm). Images were (1) slice-time corrected, (2) realigned and resliced into 3 mm isotropic voxels, (4) coregistered to the high resolution 3D anatomic volume, and (5) warped into MNI standard space using the deformations used to realign the 3D anatomical data into MNI space.

Data-based approach to RS network analysis. For the data driven analyses, an additional preprocessing step included smoothing the fMRI data with a 6 mm FWHM Gaussian kernel. The next step in the data-driven approach was done with the Group independent component analysis (ICA) of fMRI Toolbox (GIFT v3.0a; http://icatb.sourceforge.net/). ICA is a data-driven statistical analysis technique that yields independent components (ICs), which isolate sources of variance within the data. Each estimated IC represents a group of brain regions with a unique pattern of synchronized neural activity (i.e., time course) and can be conceptualized as an RSN (Calhoun et al., 2004). The GIFT IC estimation procedure occurs in three stages: (1) reduction of data dimensionality, (2) estimation of group signal sources and reduction of mutual information among those sources, and (3) back reconstruction of group-level ICs to a single-subject level. We estimated 25 IC, from which we used a spatial matching procedure to select the 5 IC that best fit the previously published RSN templates for the DMN, SMN, SN, and the RFPN, LFPN (Smith et al., 2009). SPM12 was used to perform independent t-tests with each IC to identify significant group differences in spatial recruitment for each network. These contrasts were used to investigate group differences in the intrinsic connectivity of an RSN and brain regions that showed disrupted connectivity with the RSN. RSNs are associated with particular brain regions. For example, the posterior cingulate cortex (PCC) is a part of the DMN. Using the DMN as an example, should the DMN contrast show a cluster of voxels within the PCC as significantly different between groups, the interpretation would be disrupted intrinsic connectivity within the DMN. Alternatively, using the same DMN contrast, should a cluster of voxels within the insular cortex show significant group differences, the interpretation would be disrupted FC between the DMN and the insular cortex. To keep the familywise error rate at 5%, a permutation-based extent and cluster thresholding method was used, see Correction for Multiple Comparisons section.

Model-based approach to RS network analysis. For the model-based approach, additional preprocessing steps included spike correction to reduce the impact of artifacts using the postprocessing artifact detection tool (ART) toolbox for fMRI data (http://www.nitrc.org/projects/artifact_detect). Time points were identified as artifacts and later removed during the first-level general linear model, where the mean global signal changed by above three standard deviations (SD), translation movement exceeded 0.5 mm, or rotational movement exceeded 0.01°. The final preprocessing steps were then carried out using the FC toolbox Conn version 14 (http://www.nitrc.org/projects/conn) (Whitfield-Gabrieli and Nieto-Castanon, 2012) that implements the component-based noise correction method (CompCor) strategy for physiological and other noise source reduction, which included (1) temporal (band-pass) filtering set between 0.01 and 0.1 Hz and (2) removal of several sources of nonspecific variance by regression of nuisance variables. Those nuisance variables included (1) the signal averaged over the lateral ventricles, (2) the signal averaged over the deep cerebral white matter, (3) the six parameters obtained by motion correction, and (4) the artifact data points identified with the ART toolbox.

Selection of *a priori* seed regions is a critical step in the model-based approach. For this study, we used brain regions where there was a significant reduction in CBF identified by ASL scanning, as our seeds, see Table 2. Binary masks of clusters (identified by ASL) were created and used as our seed region of interest (ROI) and overlaid with a gray matter mask to include only gray matter voxels. The time course was then estimated by the spatial average of the BOLD signal over all voxels at each time point within each ROI. Zero-order Cross Correlation coefficients (CC) (i.e., bivariate correlation) for each ROI's time course between the ROI time course and the rest of the brain were estimated using the Conn_v14 toolbox.

Group-level differences between seed and voxel-based FC. Contrasts for each of the previously identified seed regions were created to distinguish brain regions of ME/CFS subjects showing differential connectivity from those of controls. Each contrast produced a map with a t-statistic for every voxel in the brain. These contrasts were used to identify brains regions, where the strength of FC (CC) differed between groups. The strength of FC estimates the extent to which fluctuations in BOLD signal (interpreted as brain activity) between two regions are coupled. Typically, values range from -1 to 1. Values closer to 1 (or -1) suggest greater coupling, while values closer to 0 suggest less coupling. Negative values suggest anticoupling. To keep the familywise error rate at 5%, a permutation-based extent and cluster thresholding method was used, see Correction for Multiple Comparisons section.

Correction for multiple comparisons. The statistical result of each method (i.e., data driven and seed based) was corrected for multiple comparisons using AlphaSim (see the AlphaSim command description at http://afni.nimh.nih.gov/afni/doc/manual/AlphaSim) available within AFNI (http://afni.nimh.nih.gov/afni/). AlphaSim uses an estimation of

the spatial correlation across voxels to generate clustering thresholds. We set the voxel $p_{uncorrected}$ <0.001, which is the recommended default lower limit (Woo et al., 2014). The resulting pair of uncorrected statistical threshold and cluster size threshold together then corrects a whole-brain map to a familywise error-corrected threshold of α <0.05. For the data-driven approach, a combination threshold of voxel $p_{uncorrected}$ <0.001 and cluster size of 20 voxels was considered significant, which corresponds with a corrected $p < 0.05$. For the seed-based approach, a combination threshold of voxel $p_{uncorrected}$ <0.001 and cluster size of 15 voxels was considered significant, which corresponds with a corrected $p < 0.05$. Two separate corrections were used because the estimated spatial correlation across voxels was different for the data-driven and seed-based approach. There was a higher spatial correlation within the data-driven method.

Correlations between clinical fatigue and FC. The Pearson's CC was used to characterize the relationship between fatigue and the strength of connectivity between seed regions and clusters of voxels that showed significant group differences. To extrapolate each subjects' mean strength of connectivity within a significant cluster, we used the response exploration software (REX, alpha 0.5) (available at http://www.nitrc.org/projects/rex/) (Duff et al., 2007). A similar procedure was used for the data-based method. Pearson's CC was utilized to estimate the relationship between the strength of FC within each RSN and ratings of fatigue within domains of the MFI. Analyses were performed using SPSS Statistics version 22.0 (IBM, Inc. Chicago IL). Significance levels were set at the alpha level 0.05. The appropriateness of conducting these assessments was done by visually inspecting the distribution of all variables. In addition, skewness and kurtosis values for each variable were determined to confirm that the assumption of normality was not violated. For this purpose, estimated values of skewness and kurtosis were divided by the respective standard error. Values greater than 1.96 were considered to violate normality.

Results

Subject characteristics

Thirty-six females (19 ME/CFS, 17 HC) with a mean age of 48.75 years (SD = 11.75 years) volunteered for this study. Table 1 provides descriptive data on the sample, including the five domains of fatigue identified by the MFI: general fatigue, physical fatigue, reduced activity, reduced motivation, and mental fatigue. Significant group differences were found for all fatigue measures (Table 1).

TABLE 2. BRAIN REGIONS USED AS SEEDS FOR FUNCTIONAL CONNECTIVITY ANALYSIS (MODEL-BASED APPROACH)

Seed name	X	y	z	K
RH cuneus (seed 1)	10	−84	22	64
RH parahippocampus (seed 2)	28	−52	0	29
LH lingual gyrus (seed 3)	−20	−78	4	52
RH inferior occipital gyrus (seed 4)	32	−94	0	42
RH cuneus (seed 5)	24	−64	18	37

Threshold t-statistic $p < 0.001$ and a cluster size (k) of 120 mm³.
x, y, and z coordinates are in Montreal Neurologic Institute (MNI) space.
RH, right hemisphere; LH, left hemisphere.

Resting-state CBF

Using ASL, subjects with ME/CFS demonstrated reduced rCBF at rest when compared to control subjects in several clusters located within the occipital (right cuneus, inferior occipital gyrus, and left lingual gyrus) and temporal lobes (right parahippocampal gyrus) (Table 2). No cortical regions had increased rCBF in subjects with ME/CFS compared to control subjects. There was, however, no significant difference in global CBF between groups.

Data-based FC

A group-level ICA was used for the evaluation of each of the five RSNs (Fig. 1). Compared to HC, ME/CFS was associated with disrupted intrinsic connectivity within the left frontoparietal network. More specifically, a region in the left superior frontal gyrus, which is a major node of this network, showed reduced coupling of activity with the rest of the network. ME/CFS was also associated with decreased connectivity between the SN and the left PCC; similarly, the SMN demonstrated decreased connectivity with the left anterior midcingulate cortex (aMCC) compared to HC (Fig. 1). No differences in FC were found within the DMN or RFPN (Table 3).

Data-based FC as predictor of fatigue. As all data met normalcy criteria (see Correlations Between Clinical Fatigue and FC section), Pearson's product moment correlations were used. The strength of connectivity between each of the disrupted regions was significantly correlated with self-reported fatigue ratings of the MFI, including general fatigue, physical fatigue, motivation, and mental fatigue (Table 4). The direction of all three relationships was negative, suggesting stronger FC is associated with less severe symptoms of fatigue.
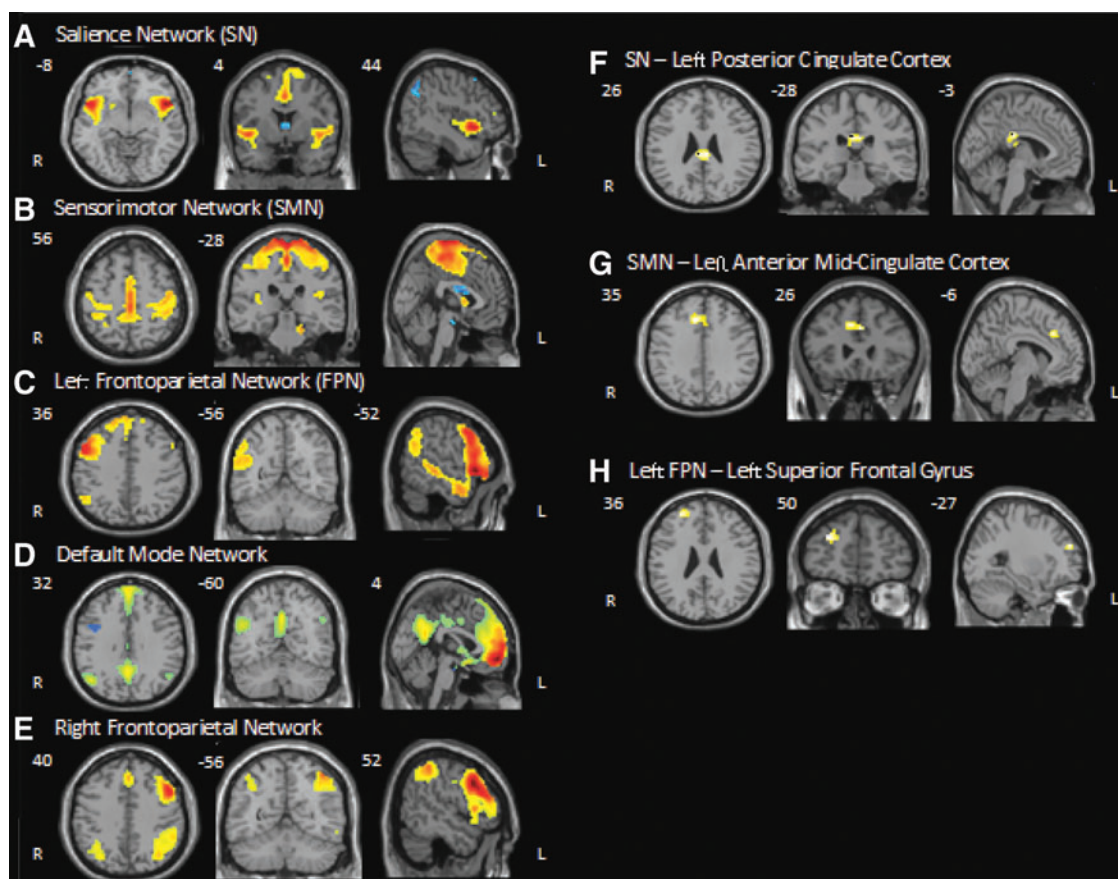
TABLE 1. DESCRIPTIVE STATISTICS OF PARTICIPANTS

	ME/CFS (n = 19)	HC (n = 17)	Group difference	p-Value
Age	52.33 (10.63)	48.75 (11.75)	3.73	0.34
General fatigue[a]	16.82 (2.10)	7.26 (2.41)	9.56	>0.01
Physical fatigue[a]	14.36 (3.05)	6.35 (2.39)	8.01	>0.01
Reduced activity[a]	12.76 (3.87)	5.65 (1.79)	7.11	>0.01
Reduced motivation[a]	11.77 (3.59)	5.79 (2.15)	5.98	>0.01
Mental fatigue[a]	13.70 (3.56)	7.38 (2.48)	6.32	>0.01

All participants were female; variables are presented as mean and standard deviation.
[a]Item of the multidimensional fatigue inventory (MFI).
HC, healthy controls; ME/CFS, myalgic encephalomyelitis/chronic fatigue syndrome.

FIG. 1. Spatial maps of resting-state networks and regional group differences in functional connectivity threshold $t > 4.0$; $k > 15$. R = Right, L = Left, x, y, and z coordinates are in Montreal Neurologic Institute space. **(A–E)** Representative group-level independent component for each of the five resting-state networks. **(F–G)** Regions of the cingulate cortex where myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) patients showed less connectivity with the salience and sensory motor networks. **(H)** Region of the left frontal gyrus where ME/CFS patients showed decreased intrinsic connectivity in the left frontoparietal network. Color images available online at www.liebertpub.com/brain

Model-based FC

Brain areas demonstrating rCBF abnormalities in ME/CFS subjects during RS ASL were used as *a priori* seeds for this analysis. Of the five seed regions, all but the right inferior occipital seed (seed 4) showed altered FC with other regions of the brain (Fig. 1). ME/CFS was associated with decreased FC between the right cuneus (seed 1) and a region in the right superior temporal gyrus; the left lingual gyrus (seed3) and regions located in the left occipital lobe and right precuneus; and the second right cuneus seed (seed 5) and regions within the right superior midfrontal gyrus and left midfrontal gyrus. Increased FC was found

TABLE 3. BRAIN REGIONS SHOWING REDUCED FUNCTIONAL CONNECTIVITY (DATA-BASED APPROACH) IN MYALGIC ENCEPHALOMYELITIS/CHRONIC FATIGUE SYNDROME SUBJECTS

RSN–brain region	x	y	z	k	t
SN—left posterior cingulate cortex	−3	−28	26	32	4.14
SMN—left anterior midcingulate cortex	−6	26	35	42	4.26
Left FPN—left superior frontal gyrus	−27	50	26	25	4.67

Threshold t-statistic of 4.0 and a cluster size (k) of 60 mm^3.
x, y, and z coordinates are in MNI space.
FPN, frontoparietal network; RSN, resting-state network; SMN, sensory motor network; SN, salience network.

TABLE 4. CORRELATIONS BETWEEN DEGREE OF FUNCTIONAL CONNECTIVITY AND SELF-REPORTED FATIGUE IN MYALGIC ENCEPHALOMYELITIS/CHRONIC FATIGUE SYNDROME SUBJECTS

	Functional connectivity		
	Intrinsic L FPN	*SN–L PCC*	*SMN—aMCC*
General fatigue[a]	−0.60[b]	−0.65[b]	−0.61[b]
Physical fatigue[a]	−0.57[b]	−0.64[b]	−0.58[b]
Reduced activity[a]	−0.51[b]	−0.58[b]	−0.50[b]
Reduced motivation[a]	−0.46[b]	−0.54[b]	−0.42[c]
Mental fatigue[a]	−0.50[b]	−0.68[b]	−0.61[b]

[a]Item of the MFI.
[b]$p < 0.01$.
[c]$p < 0.05$.
aMCC, anterior midcingulate cortex; L, left; FPN, frontoparietal network; PCC, posterior cingulate cortex.

TABLE 5. BRAIN CLUSTERS DEMONSTRATING ALTERED FUNCTIONAL CONNECTIVITY WITH SEED REGIONS IN MYALGIC ENCEPHALOMYELITIS/CHRONIC FATIGUE SYNDROME SUBJECTS

Seed—cluster	x	y	z	k	t	Direction of difference
RH cuneus (seed 1)—RH superior temporal gyrus (cluster 1)	58	−20	−4	15	4.23	ME/CFS<HC
RH parahippocampus (seed 2)—RH Inferior parietal Lobe BA 40 (cluster 1)	42	−44	40	15	4.58	ME/CFS>HC
LH lingual gyrus (seed 3)—LH occipital lobe (cluster 1)	−36	−68	−2	20	4.12	ME/CFS<HC
LH lingual gyrus (seed 3)—RH precuneus BA 7 (cluster 2)	12	−70	34	15	4.03	ME/CFS<HC
RH cuneus (seed 5)—RH superior midfrontal gyrus (cluster 1)	34	46	32	16	4.01	ME/CFS<HC
RH cuneus (seed 5)—LH middle frontal gyrus BA6 (cluster 2)	−38	−2	52	16	4.00	ME/CFS<HC

Threshold uncorrected $p < 0.001$; $k > 15$.
x, y, and z coordinates are in MNI space.
BA, Brodman area; k, cluster size; t, t-value; ME/CFS, myalgic encephalomyelitis/chronic fatigue syndrome.

between the right parahippocampus (seed 2) and the right inferior parietal lobe (Table 5).

Model-based connectivity as predictor of fatigue. As all data met normalcy criteria (see Correlations Between Clinical Fatigue and FC section), Pearson's product moment correlations were used. A significant relationship was found between the strength of connectivity and self-reports of fatigue obtained with the MFI for all disrupted seed-to-brain region pairs (Table 6). For the majority of seed-to-brain region pairs, ME/CFS subjects showed negative correlations with FC. Only for the seed-to-brain region pair (right parahippocampus to right inferior parietal lobe) a positive relationship with fatigue was observed in ME/CFS subjects.

Discussion

To identify neural correlates of self-reported fatigue, we compared patterns of resting-state FC of ME/CFS patients with age–sex-matched normal control subjects. To obtain complementary information on FC of multiple brain networks in ME/CFS, two different methods of analysis were used: (1) ICA depends on statistical associations of neural activity between brain areas and (2) a seed-based approach exploring the associations of brain activity with *a priori* determined brain areas. Our results demonstrate that ME/CFS is associated with altered resting-state FC of several brain networks,

and the degree of altered connectivity is significantly related to self-reported fatigue. Both approaches showed ME/CFS to be associated with decreased intrinsic connectivity in the LFPN and with decreased connectivity between regions in the cingulate cortex and the SMN and SN.

Neural networks encompassing multiple brain regions show synchronized activity at rest (Beckmann et al., 2005; Damoiseaux et al., 2006; De Luca et al., 2006; Salvador et al., 2005; van den Heuvel and Hulshoff Pol, 2010). The FPN is one of these networks that is composed of lateral prefrontal regions and inferior parietal cortices and often shows lateralization into right and left components (Beckmann et al., 2005; Damoiseaux et al., 2006; Di and Biswal, 2015; Smith et al., 2009). This RSN is implicated in cognitive control, attention, language processing, and working memory (Lois et al., 2014) and links the bilateral insular regions and anterior cingulate cortex (Dosenbach et al., 2007). Coherent activation between the FPN and the DMN has been suggested to play an important role in salience processes of human cognition (Fox and Raichle, 2007; Gusnard et al., 2001), including mind wandering (Mason et al., 2007), goal-directed behavior (Dosenbach et al., 2007), and relating oneself to the outside world (Gusnard et al., 2001). Considerable evidence suggests that the FPN, in particular the left FPN, plays a pivotal gate-keeping role in goal-directed cognition, mediating the dynamic balance between DMN and dorsal attention networks (Spreng et al., 2013; Vallesi et al., 2015; Vincent et al., 2008). Less

TABLE 6. CORRELATION BETWEEN DEGREE OF FUNCTIONAL CONNECTIVITY AND SELF-REPORTED FATIGUE IN MYALGIC ENCEPHALOMYELITIS/CHRONIC FATIGUE SYNDROME SUBJECTS

	Functional connectivity					
	Seed 1: cluster 1	Seed 2: cluster 1	Seed 3: cluster 1	Seed 3: cluster 2	Seed 5: cluster 1	Seed 5: cluster 2
General fatigue[a]	−0.61[b]	0.61[b]	−0.62[b]	−0.46[b]	−0.59[b]	−0.66[b]
Physical fatigue[a]	−0.50[b]	0.60[b]	−0.59[b]	−0.44[b]	−0.56[b]	−0.64[b]
Reduced activity[a]	−0.39[c]	0.57[b]	−0.59[b]	−0.44[b]	−0.58[b]	−0.62[b]
Reduced motivation[a]	−0.41[b]	0.52[b]	−0.50[b]	−0.29	−0.51[b]	−0.69[b]
Mental fatigue[a]	−0.53[b]	0.44[b]	−0.57[b]	−0.40[c]	−0.57[b]	−0.46[b]

[a]Item of the MFI.
[b]$p < 0.01$.
[c]$p < 0.05$.
Seed 1: cluster 1, RH cuneus—RH superior temporal gyrus; Seed 2: cluster 1, RH parahippocampus—RH inferior parietal lobe; Seed 3: cluster 1, LH lingual gyrus—LH occipital lobe; Seed 3: cluster 2, LH lingual gyrus—RH precuneus; Seed 5: cluster 1, RH cuneus—RH superior midfrontal gyrus; Seed 5: cluster 2, RH cuneus—LH middle frontal gyrus.

FC within the left FPN, which was seen in ME/CFS patients, may be an important feature of the condition that needs more research to fully understand its role.

The SN mainly consists of the insular cortex and anterior cingulate cortex. This RSN is involved in a wide range of functions such as detection of salient stimuli, interoception, audition, pain, deception, music, and classical conditioning (Borsook et al., 2013; Kucyi and Davis, 2015). Because of the heterogeneous range of functions, the SN has been considered a transitional network linking cognition and emotion/interoception (Lois et al., 2014; Smith et al., 2009). The SN causally influences the DMN and FPN. It also mediates the ''switching'' between activation of the DMN and of the FPN and executive control networks to guide appropriate responses to salient stimuli (Uddin, 2015). In our study, there was decreased FC of ME/CFS patients between the SN and PCC, which is a key node of the DMN. Less effective connectivity between these regions was strongly associated with more fatigue. Therefore, it appears that decreased FC between these regions may be indicative of decreased drive to guide thoughts away from self-referential experience of fatigue toward externally directed cognition.

The SMN includes primary sensory motor cortices and is associated with action and somesthesis (Lois et al., 2014). ME/CFS participants showed reduced connectivity between this RSN and the aMCC. The aMCC has a key role for cognitive aspects of movement generation, that is, intentional motor control (Hoffstaedter et al., 2014). We found that fatigue strongly predicted an inverse relationship of FC between this RSN and the aMCC. Further research is required to better understand the altered relationship of the SMN and aMCC with ME/CFS. Taking into account ME/CFS patients' impaired physical, affective, and cognitive functions and the decreased connectivity between the spatial maps of these RSNs, it can be argued that patterns of FC within or between these networks may be impaired in ME/CFS (Laird et al., 2011). We believe that our findings shed new light on the understanding of chronic fatigue across individuals and how the intrinsic connectivity of the brain is influenced by ME/CFS patients' symptoms.

Our seed-based approach was predicated on brain regions that had demonstrated altered function during ASL imaging, as evidenced by reduced rCBF. It demonstrated that the parahippocampus and occipital lobes of ME/CFS patients have altered FC. Although we are the first to report abnormal resting-state FC in patients with ME/CFS, others have described altered structure and task-related dysfunction within some of the same regions using other imaging modalities (Cook et al., 2007; De Lange et al., 2005; Okada et al., 2004). In addition, utilizing diffusion tensor imaging researchers found disrupted white matter connectivity along the right inferior fronto-occipital fasciculus in veterans with gulf war syndrome, which was marked by severe levels of fatigue and malaise (Rayhan et al., 2013). The right inferior fronto-occipital fasciculus connects posterior brain structures such as the parahippocampus and occipital lobe with anterior brain structures. Our results would support such disrupted tracts because the relationship of brain activity between posterior brain structures and intermediate structures is decreased in ME/CFS patients. Similarly, a previous study reported that multiple sclerosis patients who reported significant levels of fatigue had decreased cortical activity within the precuneus, cuneus, and middle frontal gyrus compared to MS patients without fatigue (Filippi et al., 2002).

The DMN, the most commonly studied RSN, comprises the precuneus/PCC, the medial frontal cortex, and bilateral inferior parietal regions (Fox and Raichle, 2007; Greicius et al., 2009; Gusnard et al., 2001). Activity and connectivity of the DMN have been linked to central processes of human cognition, including integration of cognitive as well as emotional brain activity (Greicius et al., 2009), monitoring of the environment (Gusnard et al., 2001), and mind wandering (Mason et al., 2007). Abnormalities of DMN connectivity have been reported in attention deficit disorder, multiple sclerosis, and Alzheimer's disease, all of which demonstrate overlapping clinical features with ME/CFS, including attention and memory difficulties. Surprisingly, we did not find fatigue-related DMN abnormalities. The DMN can be represented as a single IC or it can split into two or three components. Such a decomposition is frequently observed with separation into anterior and posterior networks (Uddin et al., 2009 and Laird et al., 2009). In our study, we choose the components that best fit the spatial template of a typical DMN. Retrospective examination of all components, however, showed three that resembled aspects of the DMN. The ability of networks to split into more than one component is a limitation of our applied methods and may explain why the DMN did not show altered connectivity between ME/CFS and HC groups.

Limitations

Some caution needs to be exercised when interpreting our results, as our sample only included female participants. Although ME/CFS affects more women than men, our results may not be applicable to males. Second, our model-based results are dependent on our seed selection method. While other researchers may use alternative seed regions for connectivity analyses, we empirically chose our seeds according to *a priori* detected rCBF abnormalities in ME/CFS. This approach of seed selection allowed us to focus on brain regions clearly associated with ME/CFS. Group-dependent rCBF findings provide a rationale to explore FC between those regions and the rest of the brain.

Conclusions

ME/CFS appears to be a chronic illness that affects several different brain areas and is associated with abnormal neuronal connectivity. Using two different methods of analysis, significant differences in resting-state FC were detected between ME/CFS and HC. Furthermore, these changes were significantly correlated with self-reported levels of fatigue. Additional neuroimaging studies will be necessary to better understand the contributions of these brain regions to ME/CFS.

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Author Disclosure Statement

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