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Abnormal Resting State Functional Connectivity In Patients with Chronic Fatigue Syndrome: An Arterial Spin-Labeling fMRI Study

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Abstract

Background—Myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) is a debilitating disorder characterized by severe fatigue and neurocognitive dysfunction. Recent work from our laboratory and others utilizing arterial spin labeling functional magnetic resonance imaging (ASL) indicated that ME/CFS patients have lower resting state regional cerebral blood flow (rCBF) in several brain areas associated with memory, cognitive, affective, and motor function. This hypoperfusion may underlie ME/CFS pathogenesis and may result in alterations of functional relationships between brain regions. The current report used ASL to compare functional connectivity of regions implicated in ME/CFS between patients and healthy controls (HC).

Methods—Participants were 17 ME/CFS patients (Mage=48.88 years, SD=12) fulfilling the 1994 CDC criteria and 17 age/sex matched HC (Mage=49.82 years, SD=11.32). All participants underwent T1-weighted structural MRI as well as a 6-minute pseudo-continuous arterial spin labeling (pCASL) sequence, which quantifies CBF by magnetically labeling blood as it enters the brain. Imaging data were preprocessed using SPM 12 and ASL tbx, and seed-to-voxel functional connectivity analysis was conducted using the CONN toolbox. All effects noted below are significant at $p<0.05$ with cluster-wise FDR correction for multiple comparisons.

Results—ME/CFS patients demonstrated greater functional connectivity relative to HC in bilateral superior frontal gyrus, ACC, precuneus, and right angular gyrus to regions including precuneus, right postcentral gyrus, supplementary motor area, posterior cingulate gyrus, and thalamus. In contrast, HC patients had greater functional connectivity than ME/CFS in ACC, left

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parahippocampal gyrus, and bilateral pallidum to regions including right insula, right precentral gyrus, and hippocampus. Connectivity of the left parahippocampal gyrus correlated strongly with overall clinical fatigue of ME/CFS patients.

Conclusion—This is the first ASL based connectivity analysis of patients with ME/CFS. Our results demonstrate altered functional connectivity of several regions associated with cognitive, affective, memory, and higher cognitive function in ME/CFS patients. Connectivity to memory related brain areas (para-hippocampal gyrus) was correlated with clinical fatigue ratings, providing supporting evidence that brain network abnormalities may contribute to ME/CFS pathogenesis.

Keywords

arterial spin labeling; chronic fatigue syndrome; functional connectivity; MRI

1. Introduction

Myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) is an illness characterized by persistent and severe fatigue, neurocognitive dysfunction, and sleep abnormalities [1]. Although numerous studies have examined potential causes of ME/CFS, the exact etiology is still undetermined. Increasing evidence suggests that ME/CFS related brain abnormalities, including global and localized cerebral hypo-perfusion of multiple brain areas associated with cognition and memory contribute to the pathogenesis of this illness [2 and 3 and 4 and 5]. Because the key features of ME/CFS include abnormal CNS functioning [6], increased focus has been placed on understanding the neural correlates of ME/CFS.

One important aspect of proper CNS functioning is regional cerebral blood flow (rCBF), which has been used as a surrogate of brain metabolism and neural activity [7]. Arterial spin labeling is a technique that can provide estimates of cerebral blood flow with good spatial and temporal resolution, without the limitations of radioactive tracer studies such as PET and SPECT

Functional connectivity (FC), a measure of the temporal coherence among brain regions, has been used for mapping large-scale brain networks [8]. Additionally, FC has been used to detect alterations in functional neural networks. Given that hypo-perfusion can lead to cerebral metabolic distress resulting in network plasticity, reduced rCBF may be associated with changes in FC [9].

Previous studies have demonstrated regional and global brain hypoperfusion in individuals with ME/CFS [2 and 3 and 4 and 5]. However, the clinical and functional significance of these findings have been questioned [10 and 11]. Understanding the functional network reorganization associated with ME/CFS might provide useful information for interpreting how reduced rCBF contributes to symptoms associated with hypoperfusion.

The goal of the present study was to examine the functional network changes of individuals with ME/CFS using seed-based FC during rest using arterial spin labeling (ASL). *A priori* regions of interest (ROIs) were chosen based on: 1) previous evidence demonstrating abnormal functional brain activity in ME/CFS patients, and 2) ROIs implicated in fatigue

and impaired cognitive/attentional function (i.e., clinical aspects of ME/CFS) including the: insula [12], inferior frontal gyrus (IFG) [12],, middle frontal gyrus (MFG) [13 and 14], parahippocampal gyrus (PaHcG) [13 and 15], anterior cingulate cortex (ACC) [16 and 17], angular gyrus (AG) [15], posterior cingulate cortex (PCC) [13], hippocampus [12], precuneus [13], caudate nucleus [18], and pallidum [18]. The superior frontal gyrus (SFG) was also selected as an *a priori* ROI because we have previously identified this region, along with PaHCG and ACC, as being hypo-perfused in ME/CFS patients during the resting state [5]

2. Methods

Participants

ME/CFS subjects had to fulfill the Center for Disease Control criteria for chronic fatigue syndrome (ME/CFS) [6]. ME/CFS subjects could not have a history of heart disease, chronic obstructive pulmonary disease, malignancy, or other systemic disorders including psychiatric illnesses that would confound the diagnosis of ME/CFS [19]. All subjects were recruited from University of Florida outpatient clinics or through advertising. Healthy controls (HC) were excluded if they had a history of chronic fatigue, chronic pain conditions, or mental illness. Individuals meeting entry criteria were asked to get a full night's sleep (at least 6 hours) and refrain from consuming caffeinated beverages prior to the laboratory session. They were also not allowed to consume alcohol or other psychoactive substances in the 24 hours before the study, or use any medications except vitamins. Additional exclusionary criteria included being a smoker or having any contraindications for MRI, including metal implants. Participants provided written consent prior to the collection of any information. All procedures were approved by the University of Florida Institutional Review Board and the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki.

Clinical and Affective Measures

The Florida Fatigue Questionnaire (FFQ) was applied to all study subjects. It has two domains, consisting of ratings of fatigue (VAS) and negative emotions related to chronic fatigue (VAS). The FFQ was only used to characterize the study subjects during the screening phase of the study. Prior to brain scanning, clinical fatigue, pain, depression, and anxiety were assessed using mechanical visual analog scales (VAS; Price et al., 1994). Each scale was anchored on the right by "no fatigue/pain/depression/anxiety at all" and on the left by "the most intense fatigue/pain/depression/anxiety imaginable". Perceived physical and role function were also assessed using VAS ranging from 0 ("no function") to 100 ("no impairment in function"). VAS measures ranged from 0–10 and were rescaled to 0–100 by multiplying each value by 10, if necessary. Additionally, each participant completed the Pennebaker Inventory of Limbic Languidness (PILL), which is a 54-item questionnaire designed to measure tendency to notice various physical symptoms and sensations [20].

Image Acquisition

Imaging data were collected using a whole body Phillips Achieva 3T MRI scanner and a 32-channel head coil. All participants were placed in the scanner head first in the supine

position. During each scanning session, participants completed a T1-weighted structural MRI scan, as well as a resting scan using a pseudo-continuous ASL protocol (i.e., pCASL). The whole brain structural images were acquired using a three-dimensional (3D) T1-weighted magnetization-prepared rapid gradient-echo (MP-RAGE) sequence with a field-of-view (FOV) of 240 mm, in-plane resolution of 1mm × 1mm, 176 contiguous sagittal slices of 1mm thickness, and TR/TE/□= 7.2ms/3.2ms/8°. The total acquisition time was 4 min 34 seconds. The ASL data were acquired using a two-dimensional (2D) pseudo-continuous arterial spin labeling (pCASL) technique [21 and 22]. with a field-of-view (FOV) of 230 mm, in-plane resolution of 3.2mm × 3.2mm, 20 axial slices of 6mm thickness, 1mm inter-slice gap, and TR/TE/□= 4s/11ms/90°. Arterial spin labeling was applied at a plane which was 30.5 mm inferior to the lowest imaging slice with a labeling during of 1500ms, and a post labeling delay time of 1800 ms. The duration of the resting state ASL scan was 6 minutes, producing 45 pairs of control and tag images.

The structural MRI scan required 4 minutes 34 seconds. The duration of the resting ASL scan was 6 minutes 24 seconds, producing 45 pairs of control and tag images.

Preprocessing Protocol

Functional imaging data processing and analyses were performed using MATLAB 2015a (MathWorks, Natick, MA, USA), SPM12 (Wellcome Department of Cognitive Neurology, UK), and ASLtbx [23]. To avoid detection of spurious motion artifacts, label and control images were motion corrected independently [23 and 24 and 24]. The functional images were then co-registered to the T1 images and spatially smoothed with a 6 mm full-width-half-maximum (FWHM) kernel to decrease noise for subsequent image subtraction. Each tag and control pair was subtracted to create 45 perfusion-weighted images. These images were used to create quantified maps of CBF using the software ASLtbx [23]. In the current study, CBF was quantified as ml/100g/min using the equation described in (Equation 1) [23]. Equation 2 contains the parameters utilized for the calculation of CBF values for this study. Four-dimensional CBF images were masked to remove out-of-brain voxels and normalized to the MNI template in SPM12.

$$f=\frac{\Delta M\,\lambda R1a\,exp\,(\omega R_{1a})}{2M_0\alpha}[1-\exp(-\tau R_{1a})]^{-1} \quad \text{Equation 1}$$

$$f=\frac{60*100\Delta M\,\lambda R_{1a}\,exp\,(1800R_{1a})}{2M_0.85}[1-\exp(-1500R_{1a})]^{-1} \quad \text{Equation 2}$$

where ΔM is the perfusion difference image, λ= 0.9 ml/g is blood/tissue water partition coefficient, R_{1a} = 1/1650 (1/ms) is longitudinal relaxation rate of blood at 3T, α= 0.85 is tagging efficiency, is equilibrium magnetization of the brain, ω= 1800 ms is post-labeling delay, and τ = 1500 ms is duration of the labeling RF pulse train.

Functional Connectivity Analysis

FC analyses were completed using CONN [25]. Prior to correlation analysis, average signals from white matter and the ventricles were removed from the data using linear regression. This step reduces spatial correlations resulting from physiological noise. Specifically, the CBF signal in the ventricles reflects cardiac-induced signal fluctuations while the CBF signal in the white matter reflects the respiratory cycle [26]. CBF volumes were filtered using a low pass (< .07 Hz) filter.

Then seed-to-voxel FC analysis was performed using each *a priori* ROI as a seed (insula, IFG, MFG, SFG, PaHcG, ACC, AG, PCC, hippocampus, precuneus, caudate nucleus, and pallidum). This analysis produced Fisher's r-to-z transformed correlation maps for each participant and seeds that subsequently were subjected to independent T-tests. Type I error was controlled through the use of cluster-level false discovery rate (FDR) correction ($p<.05$). FC values (mean z-scores) for significant clusters were extracted using the REX toolbox.

Statistical Analysis

SPSS 22 was used for all statistical analyses (IBM Corp., Armonk, NY, USA). Following descriptive statistics for demographic variables, characterization of the relationship between individuals' FC values (i.e., seed-to-cluster z-scores) and relevant clinical and psychosocial factors was conducted using nonparametric (Spearman's rho) correlation matrices.

3. Results

Demographics and Psychosocial Variables

17 women meeting CDC criteria for chronic fatigue syndrome (CFS; [6] and 17 female HC participated in this study. Participants' demographic and psychosocial variables are illustrated in Table 1. Independent t-tests indicated HC and ME/CFS were aged matched ($t_{32} = -.24$, $p = .82$). ME/CFS subjects reported significantly higher ratings for fatigue ($t_{23.21} = -9.41$, $p < .0001$), pain ($t_{17.71} = -7.79$, $p < .0001$), anxiety ($t_{17.38} = -5.64$, $p < .0001$), and depression ($t_{25.37} = -4.21$, $p < .0001$) than HC.

Functional Connectivity Analysis

ME/CFS patients demonstrated greater functional connectivity relative to HC in 5 of 21 regions used as seeds in the seed-to-voxel FC analysis (Table 2). These regions were bilateral SFG, ACC, and right AG. Compared to HC, ME/CFS participants had significantly higher FC of bilateral SFG with precuneus and postcentral gyrus, ACC with PCC and left thalamus/hippocampus, right AG with ipsilateral pre/postcentral gyri, and precuneus with bilateral supplementary motor area. HC participants also had greater FC than ME/CFS subjects in 4 of 21 regions, including ACC, left PaHcG, and bilateral pallidum (Table 3). Compared to ME/CFS subjects, HC participants showed significantly higher FC of the ACC with right insula, planum tempolare, temporal pole, putamen, and Heschl's gyrus. Left PaHcG showed greater FC with right precentral gyrus and IFG. Left pallidum showed significantly greater FC with contralateral lingual gyrus, hippocampus, fusiform cortex, and parahippocampal gyrus. Right pallidum had significantly greater FC with contralateral

occipital pole and intracalcerine cortex, as well as bilateral lingual gyri. No significant differences in FC between HC and ME/CFS subjects were detected for right PaHcG, bilateral caudate nuclei, bilateral hippocampus, left AG, bilateral MFG, or PCC. Figures 1–3 represent examples of ROIs and the locations of clusters differing significantly between ME/CFS participants and HC.

Relationship between Connectivity Measures and Clinical/Affective Measures

For brain regions (clusters) identified as different between HC and ME/CFS groups, the correlations between FC and clinical/affective measures were all significant ($p<.01$). For clusters where HC had greater FC than ME/CFS subjects, higher connectivity was associated with lower anxiety, fatigue, pain, and depression, and higher physical and role function. Where ME/CFS participants had greater FC than HC, the opposite pattern was apparent. This is likely because selection criteria (clinical symptoms), by design, differed between HC and ME/CFS subjects. The connectivity measures for the significant clusters also differed between ME/CFS participants and HC. Therefore, these correlations likely simply recapitulate the diagnosis of ME/CFS as well as differences between HC and ME/CFS subjects for the previously noted clusters.

Exploratory Spearman's correlational analyses conducted for ME/CFS participants alone revealed a significant negative correlation between left PaHcG connectivity to left postcentral gyrus and left supra-marginal gyrus and fatigue ratings ($r = -.71$, $p = .001$). In addition, right pallidum connectivity to left lingual gyrus, intracalcarine cortex, and right lingual gyrus was significantly correlated with depression ratings ($r = .50$, $p = .04$). Precuneus connectivity was also negatively correlated with depression ratings ($r = -.61$, $p = .01$). Finally, ACC connectivity to PCC, left thalamus, and left hippocampus was significantly correlated with both fatigue ($r = .56$, $p = 0.02$) and pain ($r = .52$, $p = .03$) ratings. No other correlations reached significance ($p < .05$).

4. Discussion

To our best knowledge, this is the first resting state FC study of ME/CFS patients in general and the first one using ASL. Previous investigations of CBF using different blood flow measurements, including ASL have repeatedly demonstrated global or regional hypoperfusion in ME/CFS patients [3 and 4 and 5 and 27]. We have recently reported decreased rCBF in ME/CFS patients at rest and during an exhaustive cognitive task [5]. Because the significance of this hypoperfusion for ME/CFS is unclear [10], additional studies appeared to be indicated to determine the functional relationships between abnormal rCBF and cerebral function Therefore the present study examined the functional neural networks of ME/CFS patients and their association with brain regions demonstrating abnormal rCBF during ASL, as well as with regions relevant for neurocognitive, motor, and affective functioning.

Results of our FC analyses showed abnormal connectivity patterns of ME/CFS patients for several brain regions, suggestive of functional network reorganization, including areas involved in memory (left parahippocampal gyrus), motor (bilateral pallidum), mood (ACC), and higher-order neurocognitive functions (ACC, AG, and SFG). An area of particular

interest that showed altered connectivity in ME/CFS was ACC. The ACC has been a substantial focus of interest and is known to play a critical role in higher-order neurocognitive functions, especially attention (Petersen and Posner, 2012). Furthermore, previous studies have demonstrated abnormal patterns of activation in ACC in ME/CFS patients [17]. Along with the anterior insula, the ACC has been described as a key hub of the salience network, which is associated with high-level neurocognitive control and attention [28]. Our data indicated that ME/CFS patients had significantly reduced FC between ACC and right insula. Such reduced resting-state connectivity between these regions has been associated with neuropsychological deficits [29 and 30], similar to those seen in patients with ME/CFS. Furthermore, ME/CFS patients in the present study showed increased connectivity between the ACC and hippocampus/PCC. Altered resting-state FC between these regions has been associated with Alzheimer's disease [31], and may also be relative for memory deficits of ME/CFS patients. Alternatively, increased resting-state FC between the ACC and thalamus has been previously demonstrated in patients with major depressive disorder [32]. Taken together, these findings suggest that hypoperfusion of the ACC [5] results in functional network reorganization associated with decreased neurocognitive functioning and increased affective disturbance in ME/CFS. Although the present study did not collect neuropsychological data that would confirm neurocognitive impairments in these participants, our results showed that increased connectivity of ACC with the thalamus, hippocampus, and PCC was associated with greater fatigue and pain ratings of ME/CFS patients.

Another potentially important finding was significantly lower connectivity in ME/CFS participants between left PaHCG and two distinct clusters. The PaHCG, which includes the entorhinal cortex, is involved in aspects of limbic function as well as memory retrieval and storage [33]. The first cluster encompassed broad right frontal areas including precentral gyrus, middle frontal gyrus, and inferior frontal gyrus. Notably, these anatomical regions as a whole include primary and secondary motor cortex, suggesting perturbation of the link between limbic and motor function in ME/CFS. Notably, the middle and inferior frontal gyri include structures of significant importance to higher-order cognitive function, including dorsolateral prefrontal cortex and orbitofrontal cortex. The second cluster encompassed left postcentral gyrus (i.e., primary sensory cortex) and supramarginal gyrus. Reduced connectivity between PaHCG and regions in the second cluster suggests abnormality in the link between memory functions subserved by the PaHCG and bodily sensation associated with the postcentral gyrus. Indeed, lower connectivity to this cluster was strongly correlated to higher fatigue ratings of ME/CFS participants, accounting for approximately 50% of the variance in this measure.

Finally, ME/CFS patients showed altered connectivity for both right and left SFG. Lesion studies indicate that the SFG serves a critical role in working memory function [34]. Our data suggest SFG connectivity to the precuneus is significantly higher in patients with ME/CFS than in HC. Similarly, connectivity between the precuneus and supplementary motor area, an area that contributes to the planning of movement, was also higher in ME/CFS patients than in HC. The precuneus is a medial posterior parietal structure of increasing interest in cognitive and behavioral neuroscience which has extensive cortical and subcortical connections to areas including PCC, ACC, dorsolateral prefrontal cortex,

supplementary motor area, thalamus, among others [35]. The precuneus is also an important part of the brain's default mode network [36]. Evidence suggests the precuneus is activated in a wide variety of higher-order neurocognitive processes, including motor coordination, attention, motor imagery, mental rotation, mental navigation, episodic memory retrieval, social cognition, perspective taking, and self-reflection (see Cavanna and Trimble, 2006 for review). Thus, altered connectivity between the precuneus, SFG, and supplementary motor area suggest perturbations in networks mediating motor function, working memory, and higher-order neurocognitive functions, including self-monitoring, in ME/CFS patients.

Study Strengths and Limitations

A primary strength of this study is the use of ASL, which provides a non-invasive method of directly quantifying blood flow in whole brain or in specific ROIs [26 and 26 and 37 and 38]. Furthermore, temporal fluctuation in CBF (assessed in this case using ASL) appear to correspond to underlying patterns of neuronal activity [26 and 39]. ASL has frequency characteristics that make it especially suitable for FC analyses (specifically, no significant increase in noise power at low frequencies), and has similar statistical power and reliability for FC analysis using blood-oxygenation level dependent (BOLD) contrast [26],

Although our data provide useful information regarding the functional neural correlates of global and regional hypoperfusion characteristic of ME/CFS, they are limited by the cross-sectional design of the study. Specifically, it is unclear whether the network abnormalities suggested by our data predated the diagnosis or were the result of ME/CFS. Longitudinal analysis of ASL fMRI in individuals at risk for ME/CFS may provide relevant information related to this question.

Future Directions

Results of our study suggest several promising directions for future research. The generalizability of the role of FC between regions identified in this report in the experience of fatigue should be investigated in the context of fatigue induction in both ME/CFS patients and HC. Findings that fatigue induction modulates FC measures between these regions would support the supposition that they are robust neural correlates of fatigue. In addition, investigations regarding the effect of behavioral and pharmacological treatments on FC of ME/CFS patients between seed regions and those within significant clusters are warranted. Because ME/CFS patients experience a wide range of symptoms (fatigue, pain, affective disturbance) the specificity of the FC abnormalities to ME/CFS will need additional investigations to determine the unique contributions of ME/CFS to FC.

5. Conclusions

This is the first study using ASL perfusion data to demonstrate that ME/CFS patients have abnormal FC between several brain regions subserving neurocognitive, motor, and affective-related networks, including ACC, precuneus, SFG, AG, pallidum, and PaHCG. Furthermore, the abnormal FC of ME/CFS patients correlates significantly with their symptoms, including fatigue, depression, and pain. Future studies examining the link between FC characteristics for these regions and ME/CFS are needed. In addition, the relevance of connections between

the identified regions for fatigue-related symptoms in patients suffering from other conditions (e.g., multiple sclerosis) should be investigated.

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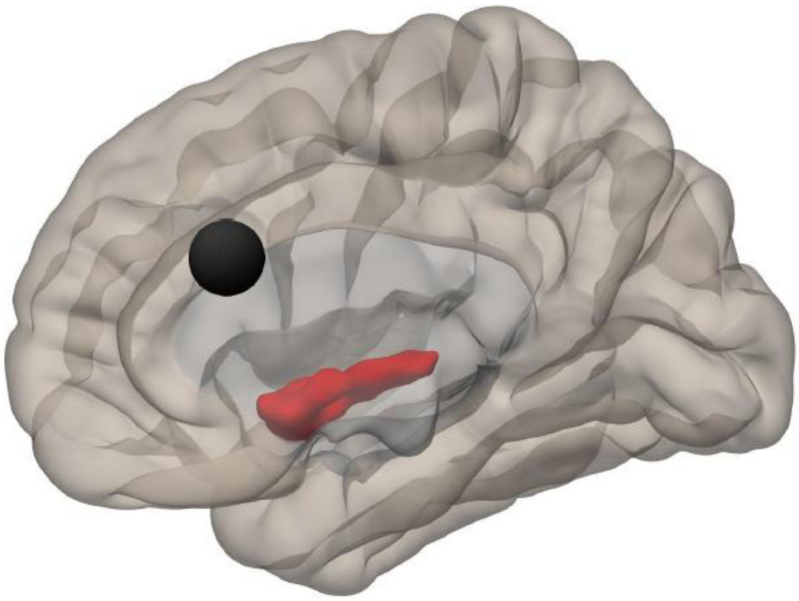

Figure 1.

Right medial view illustrating the ACC (black circle) and the location of Cluster 1 (38, −8, −4), for which connectivity was higher in HC than ME/CFS. Regions included in the cluster include right insula, right planum polare, right temporal pole, right putamen, and right Heschl's gyrus.

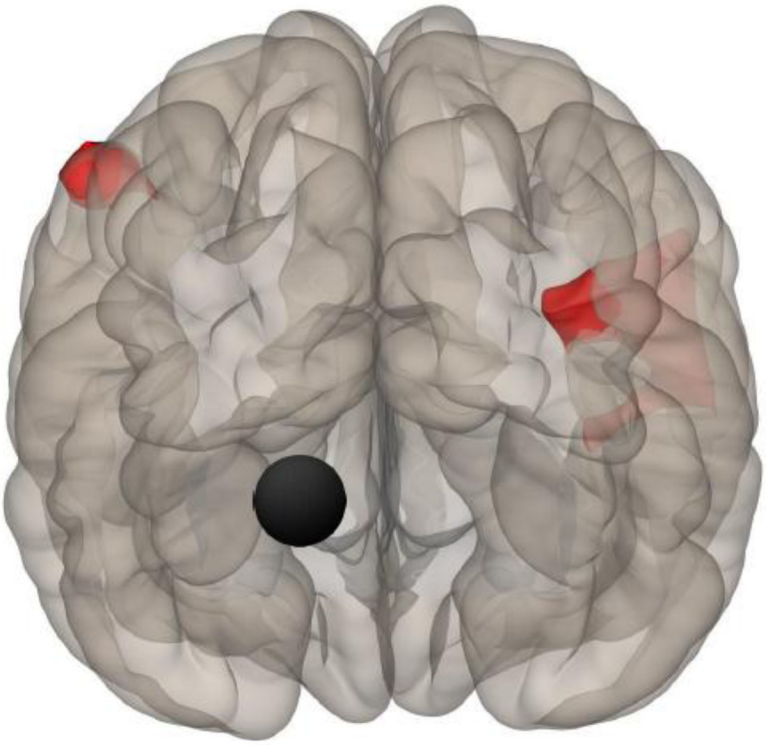

Figure 2.

Posterior view showing location of the left PaHCG (black circle) and clusters 1 (56, 6, 44) and 2 (−54, −30, 52), for which HC had higher connectivity than ME/CFS. Cluster 1 regions include right precentral gyrus, right middle frontal gyrus, and right inferior frontal gyrus (pars opercularis and pars triangularis). Cluster 2 regions include left postcentral gyrus and left supramarginal gyrus

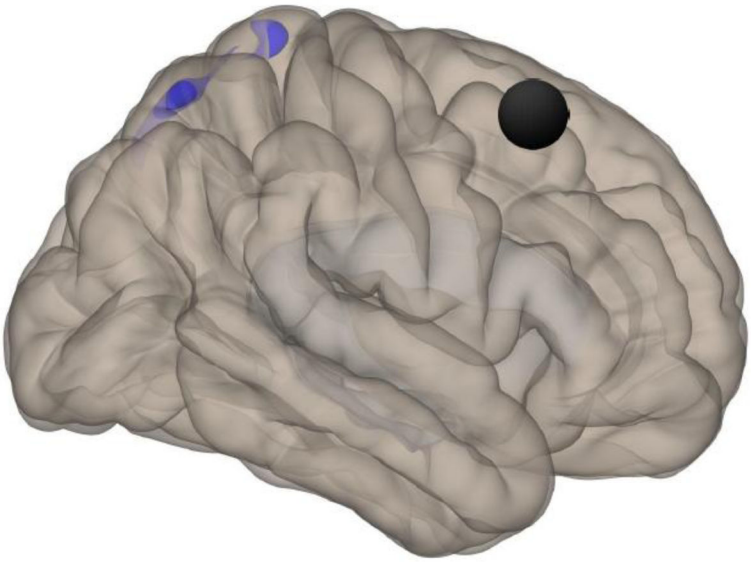

Figure 3.

Right view showing location of the right SFG (black circle) and a significant cluster (0, −72, 52) where ME/CFS showed greater connectivity than HC to a 328 voxel cluster including precuneus and right postcentral gyrus (i.e., primary somatosensory cortex).

Table 1

Demographic and Psychosocial Characteristics of Participants

	HC (n=17) Mean (SD)	ME/CFS (n=17) Mean (SD)
Age (years)	48.88 (12.00)	49.82 (11.32)
Fatigue Symptom Duration (years)	-	11.78 (9.12)
Anxiety (0–100 VAS)	2.35 (5.79)	41.29 (27.88)[a]
Fatigue (0–100 VAS)	6.94 (9.12)	54.41 (18.70)[a]
Pain (0–100 VAS)	1.65 (5.15)	44.71 (22.22)[a]
Depression (0–100 VAS)	3.94 (14.74)	34.35 (25.92)[a]
PILL Total Score	80.12 (18.44)	139.82 (29.10)[a]
Physical Function (0–100 VAS)	95.94 (6.67)	51.76 (22.57)[b]
Role Function (0–100 VAS)	92.65 (24.63)	8.82 (26.43)[b]

[a] ME/CFS > HC ($p<.05$);

[b] HC > ME/CFS ($p<.05$);

PILL: Pennebaker Inventory of Limbid Languidne

Table 2

Seed regions with increased functional connectivity in ME/CFS vs. HC

Seed Region	Cluster Coordinates	Cluster Size	Cluster Regions	Voxels in Region	% Coverage	Cluster p-value (<.05 FDR)	HC Connectivity Mean (SD)	CFS Connectivity Mean (SD)
Right Superior Frontal Gyrus	−08, −72, 52	328	Precuneus	178	3	.0008	−.13 (.16)	.19 (.20)
			Right Postcentral Gyrus	36	1			
			Not assigned or less than 1% coverage	114	-			
Left Superior Frontal Gyrus	04, −66, 60	195	Precuneus	156	3	.04	−.08 (.19)	.21 (.15)
			Not assigned or less than 1% coverage	39	-			
	02, −42, 74	172	Precuneus	41	1	.04	−.22 (.20)	.16 (.28)
			Left Postcentral Gyrus	23	1			
			Right Postcentral Gyrus	19	1			
			Not assigned or less than 1% coverage	89	-			
Anterior Cingulate Cortex	−08, −40, 10	259	Posterior Cingulate Gyrus	23	1	.01	−.34 (.17)	.15 (.26)
			Left Thalamus	22	2			
			Left Hippocampus	7	1			
			Not assigned or less than 1% coverage	207	-			
Precuneus	−06, −06, 66	465	Left Supplementary Motor Area	153	24	.0004	.01 (.17)	.29 (.12)
			Right Supplementary Motor Area	85	12			
			Left Superior Frontal Gyrus	49	2			
			Right Precentral Gyrus	29	1			
			Right Superior Frontal Gyrus	14	1			
			Not assigned or less than 1% coverage	125	-			
Right Angular Gyrus	28, −26, 78	361	Right Precentral Gyrus	117	3	.004	−.07 (.18)	.25 (.20)
			Right Postcentral Gyrus	69	2			
			Not assigned or less than 1% coverage	175	-			

Table 3

Seed regions with reduced functional connectivity in ME/CFS vs. HC

Seed Region	Cluster Coordinates	Cluster Size	Cluster Regions	Voxels in Region	% Coverage	Cluster p-Value (p <.05 FDR)	HC Connectivity Mean (SD)	CFS Connectivity Mean (SD)
Anterior Cingulate Cortex	38, −8, −4	723	Right Insula	303	23	<.0001	.38 (.11)	.09 (.14)
			Right Planum Polare	106	28			
			Right Temporal Pole	79	3			
			Right Putamen	58	7			
			Right Heschl's Gyrus	19	7			
			Not assigned or less than 1% coverage	158	-			
Left Parahippocam pal Gyrus	54, 6, 44	1163	Right Precentral Gyrus	373	9	<.00001	.21 (.11)	−.01 (.10)
			Right Middle Frontal Gyrus	365	13			
			Right Inferior Frontal Gyrus (*pars opercularis)*	85	12			
			Right Inferior Frontal Gyrus (*pars triangularis)*	16	3			
			Not assigned or less than 1% coverage	324	-			
	−54, −30, 52	251	Left Postcentral Gyrus	154	4	.002	.18 (.11)	−.02 (.11)
			Left Supramarginal Gyrus	73	8			
			Not assigned or less than 1% coverage	24	0			
Left Pallidum	38, −36, −10	448	Right Hippocampus	82	12	.0006	.17 (.15)	−.13 (.17)
			Right Lingual Gyrus	41	2			
			Right Fusiform Cortex	34	5			
			Right Parahippocam pal Gyrus	11	3			
			Not assigned or less than 1% coverage	280	-			
Right Pallidum	0, −62, 6	286	Left Lingual Gyrus	104	7	.03	.15 (.15)	−.09 (.12)
			Left Occipital Pole	45	2			
			Left Intracalcarine Cortex	41	6			
			Right Lingual Gyrus	25	1			
			Not assigned or less than 1% coverage	76	-			