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Functional brain connectivity of remembered fatigue or happiness in healthy adults: Use of arterial spin labeling

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ABSTRACT

Introduction: Chronic fatiguing illnesses like cancer, multiple sclerosis, chronic fatigue syndrome, or depression are frequently associated with comorbidities including depression, pain, and insomnia, making the study of their neural correlates challenging. To study fatigue without such comorbidities, functional connectivity (FC) analyses were used in healthy individuals to study brain activity during recall of a fatiguing event inside the MRI scanner. A positive mood induction served as control condition. *Method*: Using SPM8 and the CONN toolbox, FC was tested using seed- and independent component- based (ICA) analyses. Differences in the FC correlations between seed-to-voxel and ICA clusters between conditions were assessed with permutation testing. *Results*: 17 participants (59% women) achieved mean (*SD*) in-scanner fatigue VAS ratings of 31.85 (20.61). Positive mood induction resulted in happiness ratings of 46.07 (18.99) VAS. Brain regions where alterations in FC correlated with fatigue included the globus pallidum, left lateral occipital cortex, and cuneus. FC of happiness involved the parahippocampal gyrus, both supplemental motor areas, as well as right superior frontal gyrus. Using data-driven ICA, we identified an intra-cerebellar network where several regions were significantly associated with fatigue, but not happiness ratings. Results of permutation testing provided evidence that the detected clusters correlated differentially with self-reported fatigue and happiness. *Conclusions*: Our study suggests that functional interactions between globus pallidum and occipital structures contribute to experimental fatigue in healthy individuals. They also highlight the important role of cortico-cerebellar interactions in producing feelings of fatigue. FC of occipital structures contributed to both experimental fatigue and happiness ratings.

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Arterial spin labeling; fatigue; MRI; mood induction

Fatigue is a frequent symptom secondary to many medical conditions, including depression, heart failure, cancer, multiple sclerosis, and chronic pain (Anonymous, 2013; Alpert, Smith, Hummel, & Hummel, 2017; Brenner & Piehl, 2016; Kanbayashi & Hosokawa, 2017; Wolfe et al., 2010). Along with musculoskeletal pain and cognitive disturbance, fatigue is the primary symptom of patients with myalgic encephalomyelitis /chronic fatigue syndrome (ME/CFS) (Holgate, Komaroff, Mangan, & Wessely, 2011).

Growing evidence from our group and others implicates central nervous system abnormalities as critical components of chronic fatigue (Boissoneault, Letzen, Lai, Robinson, & Staud, in press; Boissoneault et al., 2016; Yoshiuchi, Farkas, & Natelson, 2006), although its mechanisms are only partially understood.

Several recent studies using structural or functional magnetic resonance imaging (s/fMRI) have focused on the characterization of central nervous system (CNS) abnormalities in chronic fatiguing illnesses like multiple sclerosis (MS), cancer, and ME/CFS. For instance, in multiple sclerosis, fatigue has been associated with perturbation of neuronal metabolism in the pontine reticular activating system (Zaini, Giuliani, Beaulieu, Kalra, & Hanstock, 2016), white matter dysfunction in the thalamus (Wilting et al., 2016), and lower activation in structures related to memory, motor function, somatosensation, and cognitive control during a motor task (Rocca et al., 2016). Cancer-related fatigue has been associated with perturbations in functional connectivity between regions associated with self-referential thinking (Hampson, Zick, Khabir, Wright, & Harris, 2015). Studies of ME/CFS patients have indicated that greater fatigue is associated with lower activation of basal ganglia structures during cognitive task performance (Miller et al., 2014), as well as decreased functional connectivity in fronto-parietal, salience, and sensory-motor networks (Gay et al., 2016).

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Furthermore, studies using Xenon computed tomography (CT) have identified reduced cerebral blood flow (CBF) in cortex and brainstem in ME/CFS patients (Costa, Tannock, & Brostoff, 1995; Yoshiuchi et al., 2006). Overall, these results suggest that cerebral factors may play an important role for chronic fatigue, but more specific approaches are needed for the identification of relevant CNS fatigue mechanisms.

Using arterial spin labeling (ASL), we have previously demonstrated abnormalities in resting state functional connectivity (FC) in ME/CFS, with lower connectivity between memory-related temporal structures and somatosensory structures (Boissoneault et al., 2016). In addition, abnormal connectivity between attention-, sensory-, and memory-related areas was associated with chronic fatigue intensity (Boissoneault et al., 2016). Our current work suggests that increased coherence between the hippocampus and superior parietal lobule over time during performance of a prolonged cognitive task was associated with less task-induced fatigue (Boissoneault et al., in press). Altogether, these studies suggest that fatigue may be associated with alterations in the structure and function in brain regions and networks associated with arousal, memory, attention, motor function, somatosensation, and sense of self. However, these findings were likely influenced by concomitant pain, depression, anxiety, and dysfunction as well as illness duration. Similarly to other chronic conditions like chronic pain (Wood, 2010) or depression (Stuke et al., 2016), the abnormal brain structure/function of ME/CFS patients may have developed over time and thus cannot be well characterized by cross-sectional studies. Therefore, the identification of the neuronal signature for fatigue will benefit from the presence of fatigue without pain, depression, exhaustion, etc. If similar brain networks are implicated in ME/CFS pathology and remembered fatigue, such a signature could provide guidance for mechanism-based, symptom-specific treatments for ME/CFS.

One such important control condition is "recall-induced fatigue" in healthy individuals. In this arterial spin labeling fMRI study, we induced fatigue in healthy controls by asking them to recall recent severe fatiguing events. This approach not only draws on previous strong fatigue experiences of study participants, but also asks them to re-experience such events during functional neuroimaging. Because healthy individuals lack symptoms typical of ME/CFS or related conditions like depression, pain, and anxiety, they could provide a basis from which functional and structural brain abnormalities of patients with ME/CFS or other fatiguing conditions can be characterized.

We hypothesized that experimentally induced fatigue in healthy individuals would be associated with alterations in FC between *a priori* determined brain regions previously identified as having abnormal function in ME/CFS patients. As control conditions we used happy mood induction and neutral conditions. We also performed exploratory, data-driven network analysis in order to identify additional regions that may be related to the experience of fatigue.

Method

Participants

Healthy participants were recruited from the local community via fliers and advertising. They were excluded if they reported a history of chronic fatigue, chronic pain, or mental illness. Individuals with contraindications for MRI (e.g., ferromagnetic implants, pregnancy, and claustrophobia) were also excluded. Qualifying individuals were asked to get a full night's sleep (≥6 hours), refrain from drinking caffeinated beverages on the day of the imaging session, not consume alcohol or other psychoactive substances in the 24 hours prior to the study day, and not use any medications except vitamins.

Written informed consent was obtained before the study procedures or collection of any data. The University of Florida Institutional Review Board approved all procedures, and the study protocol conformed to the ethical guidelines of the 2013 Declaration of Helsinki.

Demographic and affective measures

Participants provided demographic data and completed the Center for Epidemiologic Studies Depression scale (CES-D) (Radloff, 1977) during screening. Immediately prior to scanning, all participants also rated their overall pain, depression, anxiety, fatigue, and happiness using a visual analogue scale (VAS). These VAS ranged from "no pain/depression/anxiety/fatigue/happiness at all" to "most intense pain/depression/anxiety/fatigue/happiness imaginable" (Price, Bush, Long, & Harkins, 1994).

Fatigue and positive emotion induction protocol

A standardized protocol was used to induce fatigue or positive emotion in the laboratory. During screening sessions, participants were instructed to recall a recent severe fatigue experience and write a brief narrative about this event. Afterward, they were seated in a comfortable chair in a dimly lit room and asked to

recount their experience to a research assistant at their own pace. Participants then quietly focused on their fatiguing experience for 3 min, while providing mechanical VAS ratings of their perceived fatigue once per minute. After a 1-min break, this procedure was repeated. A similar procedure was used for inducing positive mood. The order of fatigue and positive mood induction during screening was counterbalanced across participants. In order to limit enrollment to individuals who were able to successful induce fatigue and positive emotion (i.e., happiness) in this way, participants who did not report increases in fatigue and happiness of at least 3 VAS units from baseline or achieve ratings of at least 4 units during practice were excluded from further study. This procedure resulted in the exclusion of 28 participants prior to the scanning sessions. Among participants meeting study inclusion criteria, paired *t*-tests indicated that both the fatigue and positive mood induction procedures were associated with significant increases from pre- to post-induction ($ts > 8.51$, $ps < .0001$, Cohen's $d > 2.32$).

During scanning sessions, subjects quietly recalled the same fatiguing and positive emotion-related events for two consecutive 184-s ASL fMRI runs per condition.

Fatigue and happiness ratings during MRI

During each run, participants rated their fatigue or happiness on an electronic VAS (0–100) anchored from "No fatigue/Not at all happy" to "Most fatigue imaginable/Happiest imaginable" using an MR-safe scroll wheel in their right hand. The order of fatigue and positive mood induction runs was counterbalanced across subjects, resulting in two orders: Fatigue–Positive Mood–Neutral and Positive Mood–Fatigue–Neutral. During the two "neutral" runs, participants were instructed to slowly move a VAS to the right over the course of each run to control for movement related to the rating of fatigue/happiness. A marker was placed on the VAS based on the average ratings from each subject's average fatigue and happiness runs. For instance, if a subject reported an average in-scanner fatigue rating of 40 during their two fatigue induction runs, a marker was placed at 60 on the neutral VAS, and the participant moved the marker slowly to 100. VAS were presented using PsychoPy (Peirce, 2007, 2009) running on a Dell Latitude laptop (Dell Inc., Round Rock, TX, USA).

Image acquisition

Neuroimaging data was collected using a whole-body Philips Achieva 3T scanner and a 32-channel head coil (Koninklijke Philips N.V., Amsterdam, Netherlands). Scanning sessions included a T1-weighted structural MRI scan and 6 scans utilizing pseudo-continuous arterial spin labeling protocols (pCASL) (Dai, Garcia, deBazelaire, & Alsop, 2008; Wu, Fernández-Seara, Detre, Wehrli, & Wang, 2007).

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 1 mm × 1 mm, 176 contiguous sagittal slices of 1 mm thickness, and TR/TE/flip angle = 7.2 ms/3.2 ms/8°. ASL data were acquired using a two-dimensional (2D) pCASL technique with a field-of-view (FOV) of 230 mm, in-plane resolution of 3.2 mm × 3.2 mm, 20 axial slices of 6 mm thickness, 1-mm interslice gap, and TR/TE/flip angle = 4 s/11 ms/90°. Arterial spin labeling was applied at a plane 30.5 mm inferior to the lowest imaging slice with a labeling time of 1500 ms and a post-labeling delay time of 1800 ms. Structural MRI required 4 min, 34 s. Each of the 6 ASL scans (2 per task condition) required 3 min, 4 s. resulting in 23 pairs of control and tagged images per run (46 per condition).

Preprocessing protocol

Imaging data processing and analyses were performed using MATLAB 2015a (MathWorks, Natick, MA, USA), SPM8 (Wellcome Department of Cognitive Neurology, UK), and ASLtbx (Wang et al., 2008). pCASL scans were corrected for subject motion using a rigid body 6-parameter algorithm included in SPM8. Tagged and control images were motion-corrected independently in order to avoid arterial spin labeling being detected as movement (Wang, 2012; Wang et al., 2008). Functional images were then coregistered to the T1-weighted images and spatially smoothed with a Gaussian filter of 6 mm full-width-half-maximum (FWHM) kernel. Tagged and control pairs were subtracted to create 23 perfusion-weighted images. Perfusion-weighted images were converted to CBF maps using ASLtbx, quantified as ml/100 g/min (Wang et al., 2008), using previously published parameters (Boissoneault et al., 2016). Four-dimensional CBF images were normalized to native space, warped to the SPM12 MNI template, and masked to remove out-of-brain voxels. Four dimensional CBF images were visually inspected for artifacts, and movement parameters/outliers were characterized using the ART toolbox. Three participants with excessive motion were excluded.

Functional connectivity analyses

The CONN toolbox (Whitfield-Gabrieli & Nieto-Castanon, 2012) was used for FC analysis. Prior to FC analyses, the signal from white matter and the ventricles was removed from the data using the CompCor algorithm (Behzadi, Restom, Liau, & Liu, 2007). This step reduces spurious spatial correlations due to physiological noise. This is relevant because the CBF signal in the ventricles and the white matter is affected by the cardiac and the respiratory cycle, respectively (Fernández-Seara et al., 2015). This approach is at least as effective for ASL data as denoising with physiological data (Behzadi et al., 2007). CBF time series signals were filtered using a low pass (<.07 Hz) filter.

Next, seed-to-voxel FC analysis, including in-scanner fatigue or happiness ratings as covariates of interest using 14 *a priori* identified regions, was conducted to identify functional networks related to the experience of fatigue and/or happiness (i.e., between-subjects contrast: subject, VAS rating: contrast [0 1]; between-conditions contrast: fatigue/happy, neutral: contrast [1–1]; between-sources contrast: ROI [1]). These analyses used the neutral condition as a baseline condition to control for movement related to the rating of fatigue and/or happiness. Regions of interest included superior parietal lobule, angular gyrus, precuneous, parahippocampal gyrus, hippocampus, thalamus, inferior frontal gyrus, anterior cingulate, superior temporal gyrus, caudate, cuneus, insula, posterior cingulate, and globus pallidus. These regions were identified based on previous studies indicating functional abnormalities in ME/CFS patients (Boissoneault et al., in press, 2016). Bilateral pairs of ROIs used as seeds were derived from the main effect of the left- and right-hemisphere time series for each region. As in previous studies, ROIs were defined as 10 mm spheres based on the Harvard-Oxford cortical and subcortical structural atlases (Desikan et al., 2006; Frazier et al., 2005; Goldstein et al., 2007; Makris et al., 2006). Type I error was controlled using cluster-size-based familywise error (FWE) correction, with the significance threshold adjusted using Bonferroni correction ($p_{FWE} < .0036$; .05/14). FC values (mean *z*-scores) for significant clusters were extracted using the REX toolbox.

Exploratory group-level independent component analysis (ICA) using the CONN toolbox was also conducted to identify networks of brain regions with unique CBF time courses that may be associated with fatigue and/or happiness ratings. This involved the application of the fastICA algorithm to dynamic volumes concatenated across subject and condition in order to identify independent spatial components and back-projection of these components to individual subjects, resulting in maps of regression coefficients representing FC between the IC network and every voxel in the brain (see Calhoun, Adali, Pearlson, & Pekar, 2001 for details). Twenty independent components (ICs) were identified. Noise components were identified by two authors (JB and LS) through visual inspection (e.g., components largely overlapping CSF), resulting in the exclusion of 2 out of 20 ICs from further consideration. Each of the remaining 18 ICs was used in analyses as described above (i.e., between-subjects contrast: subject, VAS rating: contrast [0 1]; between-conditions contrast: fatigue/happy, neutral: contrast [1–1]; between-sources contrast: IC [1]). As before, Type I error was controlled using cluster-size-based familywise error (FWE) correction with Bonferroni-corrected significance thresholds ($p_{FWE} < .0028$; i.e., .05/18). FC values (regression coefficients) for significant clusters were then extracted using the REX toolbox.

Differences in the correlation between FC of detected seed-to-voxel (*a priori* ROI analysis) and network-to-voxel (exploratory ICA) clusters between conditions were tested using permutation testing. In this procedure, individual FC values for each cluster as well as behavioral ratings during fatigue and positive mood induction were randomly permuted 1000 times (i.e., sampling without replacement). For each iteration, the Pearson's r correlation coefficient between FC for each cluster and fatigue/happiness ratings was calculated. Then, the difference in *r*-values for fatigue and happiness was calculated. This resulted in a null distribution of differences in correlation between fatigue and positive mood ratings for each cluster. Differences in correlation between conditions for each cluster exceeding the 95th percentile of the null distribution were considered statistically significant.

Results

Participant characteristics and behavioral ratings

The average (*SD*) age of the subjects was 22.4 (5.9) years, and 59% of participants were women. Average (*SD*) CES-D scores were 5.88 (5.63). Across participants, the fatigue induction procedure resulted in mean (*SD*) in-scanner fatigue VAS ratings of 31.85 (20.61). Positive mood induction resulted in mean in-scanner happiness ratings of 46.07 (18.99). Paired *t* tests comparing pre-scan fatigue and happiness ratings with in-scanner ratings indicated that both ratings increased significantly from baseline levels [$t(16) = 4.98$, $p < .001$, Cohen's $d_z = 1.35$;

Table 1. Participant behavioral ratings.

	M (*SD*)
Pre-Practice Fatigue Rating (0–100 VAS)	5.88 (7.44)
Post-Practice Fatigue Rating (0–100 VAS)	52.41 (13.95)
Pre-Practice Happiness Rating (0–100 VAS)	6.57 (11.57)
Post-Practice Happiness Rating (0–100 VAS)	53.75 (17.33)
Pre-induction Fatigue (0–100 VAS)	8.81 (9.99)
Post-induction Fatigue Rating (0–100 VAS)	31.86 (20.61)
Pre-induction Happiness (0–100 VAS)	26.59 (25.38)
Post-induction Happiness Rating (0–100 VAS)	46.07 (18.99)

$t(16) = 5.53$, $p < .001$, Cohen's $d_z = 1.48$, respectively] (Table 1).

Functional connectivity analysis

Analyses indicated a positive relationship between FC of globus pallidus to left lateral occipital cortex and cuneus and in-scanner fatigue ratings surviving cluster-wise FWE correction as well as Bonferroni correction ($r_{fatigue} = .87$; Figure 1; Table 2). Permutation testing indicated the relationship between FC between these structures and fatigue was significantly greater than for happiness ratings during positive mood induction ($p < .05$; Figure 2).

FC for the other 13 *a priori* ROIs was not significantly associated with fatigue ratings.

Happiness ratings during positive mood induction were significantly associated with FC for 3 seed regions (Table 2). Greater FC between parahippocampal gyrus and a cluster including right supplemental motor area, right superior frontal gyrus, and left supplemental motor area was associated with greater happiness ratings ($r = .88$, $p_{FWE} < .0001$) (Figure 3). Similarly, greater FC between inferior frontal gyrus and a cluster including right fusiform gyrus, right cerebellum, and right lingual gyrus was positively associated with happiness ratings ($r = .87$). Conversely, greater connectivity between cuneus and a cluster including left occipital pole, left fusiform gyrus, and left lingual gyrus was associated with lower happiness ratings ($r = -.83$). Permutation testing indicated the relationships between FC of the parahippocampal gyrus and cuneus, and happiness ratings were significantly different from fatigue ($p < .03$). However, the correlation between inferior frontal gyrus connectivity to right occipital structures did not differ significantly between the fatigue and positive mood induction conditions ($p = .13$).

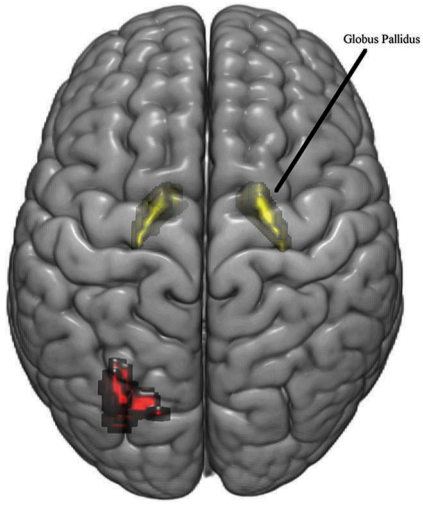

Figure 1. FC analysis indicated that greater connectivity between globus pallidus and a large cluster encompassing left lateral occipital cortex and cuneus was significantly positively associated with VAS ratings of fatigue. This relationship was not detected for any other *a priori* region of interest. Notably, cluster significance survived Bonferroni correction for familywise error ($\alpha_{Bonferroni} = .05/14 = .0036$).

Exploratory ICA

FC of one IC, the spatial extent of which largely overlapped with the cerebellum, was identified as significantly correlating with participants' in-scanner fatigue ratings (Figure 4). Analyses indicated several clusters where greater FC between this cerebellar network was associated with higher in-scanner fatigue ratings, including (a) right frontal pole and right inferior frontal gyrus; (b) anterior cingulate cortex; and (c) left hippocampus, parahippocampal gyrus, and fusiform cortex. Clusters where lower connectivity with this cerebellar network was associated with higher fatigue ratings included (d) right occipital pole; (e) left middle frontal gyrus and paracingulate gyrus; and (f) right cerebellum and brainstem (Table 3). No other IC correlated with fatigue ratings, and no ICs correlated with happiness ratings. Permutation testing indicated the relationships between FC of the cerebellar IC with all detected clusters, and fatigue ratings were significantly different from happiness ($p < .05$).

Discussion

We identified several regions of interest where alterations in FC correlated significantly with self-report of fatigue (globus pallidum to L lat. occipital cortex, cuneus) and happiness (parahippocampal gyrus to R and L suppl. motor areas, R sup. frontal gyrus). Using data-driven ICA, we also identified an intra-cerebellar network where FC with several brain regions was significantly associated with fatigue but not with happiness ratings. Critically, with only a single exception, results of permutation testing provided evidence that

Table 2. Seed regions/clusters where FC predicted fatigue and happiness ratings.

Condition	Seed region	Cluster MNI coordinates (*x, y, z*)	Cluster regions	Voxels in region	Coverage (%)	Cluster *p* (<.0036 FWE)	Connectivity *M* (*SD*)	Correlation with fatigue/happiness (*r*)
Fatigue	Globus pallidus	−26, −78, 26	Left lateral occipital cortex	246	5	.0019	−.09 (.26)	.87
			left cuneus	14	2			
			not assigned or less than 1% coverage	44				
Positive mood	Parahippocampal gyrus	12, −2, 66	Right Supplemental motor area	283	40	.00007	−.006 (.24)	.88
			Right superior frontal gyrus	62	2			
			Left supplemental motor area	46	7			
			Unlabeled or less than 1% coverage	114	—			
	Inferior frontal gyrus	28, −66, −18	Right fusiform gyrus	179	20	.001	−.07 (.25)	.87
			Right cerebellum	81	5			
			Right lingual gyrus	36	2			
			Right fusiform gyrus	29	4			
			Unlabeled or less than 1% coverage	6				
	Cuneus	−14, −88, −8	Left occipital pole	98	4	.0008	.05 (.25)	−.83
			Left fusiform gyrus	82	9			
			Left lingual gyrus	30	2			
			Unlabeled or less than 1% coverage	115				

the detected clusters correlated differentially with self-reported fatigue and happiness.

Of great interest is the correlation between higher FC of glob. pallidum, a component of the basal ganglia, to a cluster including left lateral occipital cortex and cuneus and greater fatigue ratings during experimental fatigue induction. Lateral occipital cortex and cuneus are each involved in visual processing. These structures have been previously implicated in ME/CFS symptomatology: in a resting-state ASL fMRI study, we found disrupted FC between pallidum and occipital structures in ME/CFS patients that correlated with ratings of negative affect (Boissoneault et al., 2016). Similarly, Miller et al. found that pallidum activation during a cognitive task was lower among ME/CFS patients than HC (Miller et al., 2014). Lower gray-matter volume in left lateral occipital cortex has also been reported, suggesting that functional perturbations related to ME/CFS may be reflected in brain structure (Puri et al., 2012). These converging results highlight the possibility that the pallidum and occipital cortices each play an important role in the normal experience of fatigue, and that ME/CFS symptomatology may be underpinned by dysfunction within and between these structures.

Results of FC analyses and subsequent permutation tests suggested that the functional networks subserving

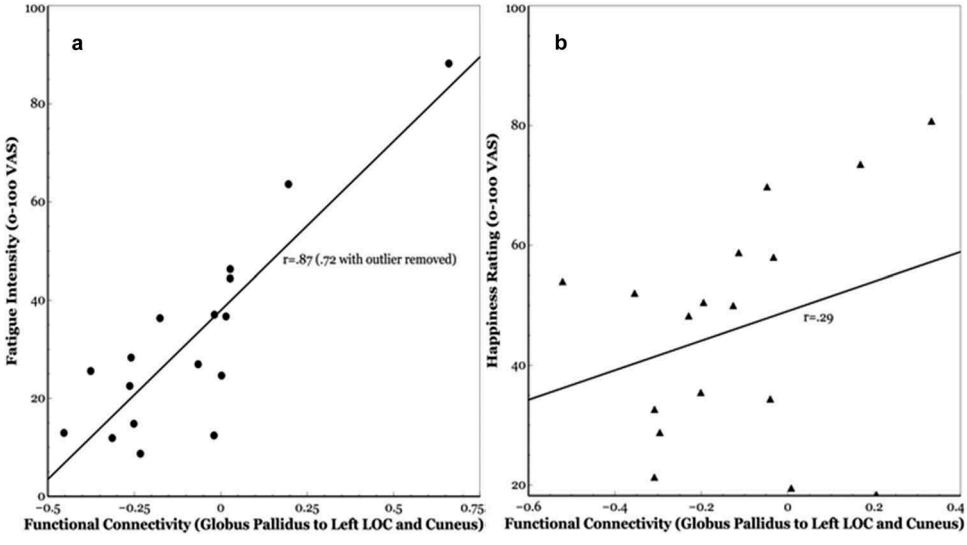

Figure 2. Greater FC between globus pallidus and left lateral occipital cortex in the FI condition was strongly correlated with (a) fatigue intensity but not (b) happiness. Permutation testing indicated this difference in association was statistically significant ($p < .05$).

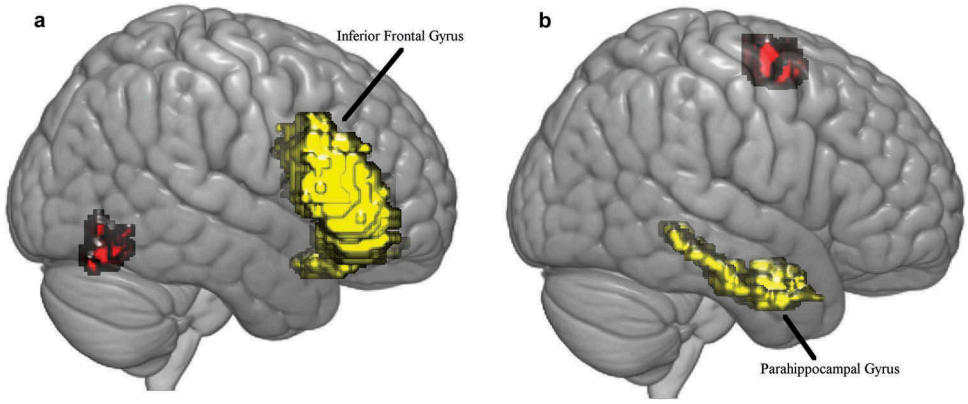

Figure 3. Greater connectivity between (a) inferior frontal gyrus and a 331-voxel cluster including right fusiform gyrus, right cerebellum, right lingual gyrus, and right fusiform gyrus; and (b) parahippocampal gyrus and a 505-voxel cluster encompassing right supplemental motor area, right superior frontal gyrus, and left supplemental motor area each predicted greater happiness ratings during positive mood induction. Permutation testing indicated that the correlation between FC of parahippocampal gyrus and frontal structures was significantly greater for positive mood than fatigue induction ($p < .05$).

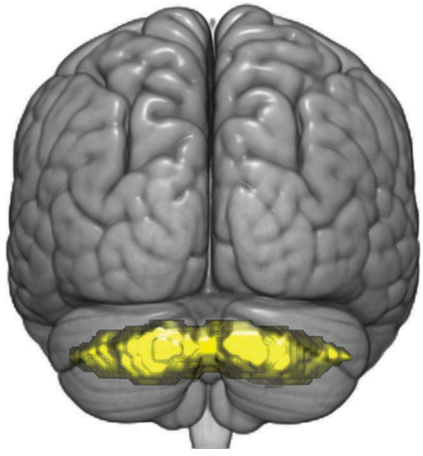

Figure 4. Spatial extent of an ICA-derived cerebellar network (threshold set at $z > 4$ for visualization purposes) where functional connectivity with several clusters including occipital, temporal, and frontal structures was associated with in-scanner fatigue ratings.

feelings of fatigue and happiness resulting from our experimental fatigue induction were largely distinct from one another. Significant correlations were noted between FC of parahippocampal gyrus with bilateral supplemental motor area and right superior frontal gyrus; inferior frontal gyrus with right fusiform gyrus, cerebellum, lingual gyrus, and fusiform gyrus; and cuneus with left occipital pole, fusiform gyrus, and lingual gyrus with happiness ratings. Thus, although FC of occipital structures contributed to both experimental fatigue and happiness ratings, the interacting regions differed between conditions. We speculate that these occipital structures, which may play a role in the experience of remembered fatigue- and happiness-related scenarios, interact differentially with other cortical (e.g., fusiform gyrus, lingual gyrus) and sub-cortical (e.g., pallidum) structures to produce these subjective states in healthy individuals.

Interestingly, our exploratory model-free analysis identified an independent component (IC) network spatially constrained to the cerebellum where FC with several clusters correlated significantly with experimental fatigue ratings. Positive associations between network-to-cluster FC and fatigue were found for regions including right frontal pole, anterior cingulate cortex, left hippocampus, and left parahippocampal gyrus, which collectively subserve functions related to attention, affective modulation, memory, and cognitive control (Petersen & Posner, 2012; Simons, Elman, & Borsook, 2014). Negative associations between network-to-cluster FC and fatigue were identified for right occipital pole, left middle frontal and paracingulate gyri, and right cerebellum and brainstem, structures related to visualization, memory retrieval, motor/cognitive function, and autonomic regulation (Rajah, Languay, & Grady, 2011).

Although the most common conceptualization of cerebellar function relates to its role in coordinating motor activity, evidence suggests that the cerebellum is also involved in the encoding and perception of aversive stimuli, including pain (Moulton et al., 2011). Increased frontocortical FC with cerebellum following exercise-based fatigue induction has been previously reported (Jiang, Wang, & Yue, 2016; Jiang, Oathes, et al., 2016), as well as disruptions in inferior frontal gyrus FC with a large cluster including bilateral cerebellum and vermis over the course of a fatiguing cognitive task in ME/CFS patients (Boissoneault et al., in press). Furthermore, Caseras et al. found that ME/CFS patients had greater cerebellar activation than controls while viewing

Table 3. Seed regions/clusters where FC with a cerebellar IC network predicted fatigue ratings.

Cluster MNI coordinates (*x*, *y*, *z*)	Cluster regions	Voxels in region	Coverage (%)	Cluster *p* (<.0028 FWE)	Connectivity *M* (*SD*)	Correlation with fatigue (*r*)
46, 32, 12	Right frontal pole	227	3	.0001	11.92 (37.23)	.84
	Right inferior frontal gyrus, pars triangularis	10	2			
	Unlabeled or less than 1% coverage	48	—			
−18, −12, 42	Anterior cingulate cortex	107	4	.0009	16.55 (38.18)	.82
	Unlabeled or less than 1% coverage	108	—			
−28, −34, −14	Left hippocampus	100	13	.0015	9.65 (37.13)	.92
	Left parahippocampal gyrus, posterior division	41	11			
	Left temporal fusiform cortex, posterior division	26	3			
	Unlabeled or less than 1% coverage	32	—			
10, −100, −8	Right occipital pole	208	8	.00005	−9.66 (33.50)	−.88
	Unlabeled or less than 1% coverage	99	—			
−4, 40, 30	Left middle frontal gyrus	22	1	.00015	−12.98 (34.56)	−.87
	Left paracingulate gyrus	13	1			
	Unlabeled or less than 1% coverage	237	—			
16, −42, −24	Right cerebellum 4, 5	82	13	.0009	−7.89 (34.82)	−.84
	Right cerebellum 6	67	4			
	Brain stem	36	1			
	Right temporal occipital fusiform cortex	6	1			
	Right cerebellum 3	5	3			
	Unlabeled or less than 1% coverage	16	—			

fatigue-related video clips (Caseras et al., 2008). Thus, results of our exploratory analysis add to the literature suggesting that the cerebellum may play an underappreciated role in producing the experience of fatigue.

Study strengths

We believe that the use of an experimental fatigue induction including within-subject controls for both general visualization- and motor-related brain activity (positive mood induction and neutral runs, respectively) represents a significant strength of the current study. In addition, the use of ASL fMRI for FC analysis provides advantages compared to blood-oxygenation-level-dependent (BOLD) fMRI. These benefits include lower sensitivity to motion artifacts, a more direct relationship with underlying neuronal activity, and a flat noise spectrum (Chen, Jann, & Wang, 2015; Fernández-Seara et al., 2015).

Limitations

A potential limitation of the study is that the FC analyses we employed are correlative measures that do not provide evidence of causality. Thus, directionality of the relationship between seed regions/networks and clusters cannot be determined. Application of network analysis approaches that allow causal inferences, e.g., dynamic causal modeling (Friston, Harrison, & Penny, 2003), in future studies would help to address this concern. We did not differentiate between subtypes of fatigue in this study, including physical, mental, and emotional fatigue, because of our relatively small sample size. However, evidence suggests that fatigue may be modeled best as a unidimensional construct (Michielsen, De Vries, Van Heck, Van De Vijver, & Sijtsma, 2004).

Conclusions

Taken together, results of our experimental fatigue induction suggest that functional interactions between pallidum and occipital structures may contribute to the experience of fatigue in healthy individuals. They also highlight a potentially important role of cortico-cerebellar interactions in producing feelings of fatigue. FC of occipital structures contributed to both experimental fatigue and happiness ratings. Future studies should compare the neural mechanisms underpinning experimental fatigue between healthy individuals and patients with ME/CFS.

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No potential conflict of interest was reported by the authors.

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