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Modulatory effects of cognitive exertion on regional functional connectivity of the salience network in women with ME/CFS: A pilot study

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ABSTRACT

Background: A common symptom of myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) is post-exertional malaise (PEM). Various brain abnormalities have been observed in patients with ME/CFS, especially in insular and limbic areas, but their link with ME/CFS symptoms is still unclear. This pilot study aimed at investigating the association between PEM in ME/CFS and changes in functional connectivity (FC) of two main networks: the salience network (SN) and the default-mode network (DMN).

Methods: A total of 16 women, 6 with and 10 without ME/CFS, underwent clinical and MRI assessment before and after cognitive exertion. Resting-state FC maps of 7 seeds (3 for the SN and 4 for the DMN) and clinical measures of fatigue, pain and cognition were analysed with repeated-measure models. FC-symptom change associations were also investigated.

Results: Exertion induced increases in fatigue and pain in patients with ME/CFS compared to the control group, while no changes were found in cognitive performance. At baseline, patients showed altered FC between some DMN seeds and frontal areas and stronger FC between all SN seeds and left temporal areas and the medulla. Significantly higher FC increases in patients than in controls were found only between the right insular seed and frontal and subcortical areas; these increases correlated with worsening of symptoms.

Conclusions: Cognitive exertion can induce worsening of ME/CFS-related symptoms. These changes were here associated with strengthening of FC of the right insula with areas involved in reward processing and cognitive control.

1. Introduction

Myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) is a condition characterised primarily by an intense and protracted sensation of fatigue. Although the aetiology of ME/CFS is not fully understood yet, a few symptoms have been consistently observed across the majority of patients with this condition: mild cognitive dysfunction [1], cardiac autonomic dysregulation [2], associated with both cognitive decline [3] and fatigue severity [4], and especially post-exertional malaise (PEM), considered to be a prominent feature of this disease [5]. PEM is the exhaustion caused by either physical or cognitive exertion [6] that may occur even with a delay of up to 24 h after effort [7] and is characterised by worsening of ME/CFS-related symptoms and a subsequent slow return to baseline levels.

The neurological dysfunctions associated with ME/CFS have been extensively investigated. However, currently available neuroimaging findings appear inconsistent across studies and a coherent interpretation of how neural abnormalities may explain ME/CFS symptoms is still lacking [8]. In fact, structural MRI studies have most commonly reported lower grey matter (GM) volume in patients with ME/CFS compared to healthy controls in various brain areas [9–11]. However, some studies have found no between-group differences in either global

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[12] or regional volumes [13] or even larger volumes in people with ME/CFS in limbic, insular, thalamic and orbito-frontal areas [14]. Moreover, one study has also observed increased myelin levels in the somatosensory white matter (WM) of patients with ME/CFS when compared with healthy controls, by using an uncommon but very stable T1 weighted spin-echo MRI sequence as scanning protocol [12]. The morphological [15] and metabolic alterations [16] that most significantly discriminate people with and without ME/CFS have been observed in the anterior cingulate cortex (ACC). Other brain areas where consistent structural alterations have been found in this clinical population are the medial prefrontal cortex (PFC), insula, caudate, thalamus, superior temporal gyri and midbrain. It is worth noting that many of these areas are either part of or are strongly connected with the salience network (SN), a neural system associated with interoception and assessment of stimulus salience [17].

Diminished resting-state functional connectivity (FC) across various limbic, frontal and insular areas have been consistently observed in patients with ME/CFS when compared with healthy individuals, although correlations between neurofunctional alterations and symptom severity have not been consistently detected [18–22]. Similarly, glucose hypometabolism has also been found in the ACC, ventromedial PFC and orbito-frontal cortex, although no correlations with fatigue levels have been reported [23]. A possible explanation of such inconsistencies may be due to the fact that the relationship between brain network dysfunctions and symptoms in people with ME/CFS is likely to be complex. In fact, several FC alterations between hubs of the default-mode network (DMN) have shown associations with both physical and mental health of patients with ME/CFS [22]. Some authors have also observed increases in functional coupling between cingulate, somatosensory, frontal and thalamic areas in patients with ME/CFS [18,24]. Moreover, one study has found no between-group differences in resting-state FC between pairs of selected regions of interest located in nuclei of the brainstem reticular activation system, the subiculum and the thalamic intralaminar nucleus [25].

Functional MRI studies of neurocognitive dysfunction in ME/CFS during selected cognitive operations have revealed that patients showed increased levels of brain activation relative to those detected in healthy controls especially in frontal areas in response to cognitive tasks as a compensatory response to sustain normal levels of performance [26–29]; decreased activation, however, was detected in more challenging task conditions [26,30]. This pattern of abnormalities in task-related brain activations is similar to that consistently found in ageing [31] and in the early stages of other neurological conditions, e.g. mild cognitive impairment [32] and multiple sclerosis [33]. Moreover, connectivity between different nuclei of the brainstem reticular activation system has been found to be decreased in patients with ME/CFS during cognitive performance [25]. Both increases [28] and decreases in brain activation [30] appear correlated with the levels of reported fatigue, but null findings have also been observed [26]. Although no significant differences in task-related brain activations and de-activations were observed in their study, Shan et al. [22] found that complexity of dynamic FC between DMN hubs was increased in patients during performance of the Stroop task and significantly associated with patients' health status. This finding suggested that task-related brain activity may be less coordinated across areas of the DMN and may be at the basis of some of the symptoms associated with ME/CFS.

Only a handful of MRI studies have focussed on the clarification of the neural processes associated with PEM, one of the core clinical manifestations of ME/CFS. Josev et al. [34] have found that adolescents with ME/CFS show a weakly significant drop in cognitive performance after cognitive exertion compared to controls. However, a similar decrease in FC between the anterior and posterior hubs of the DMN has been noted in patients and controls, irrespective of cognitive changes. Hence, whether a perturbation of the DMN plays a central role in determining PEM in people with ME/CFS is still unclear. On the contrary, increases in task-related brain activations in lateral parietal and cingulate cortices have been found after physical exertion in patients with ME/CFS [35]. Consistently, a machine-learning study has found that post-exercise alterations in brain activations across basal ganglia, parietal, frontal, temporal and cerebellar areas were associated with performance in a working memory task and could classify accurately people with and without ME/CFS [36]. However, such paucity of MRI findings on PEM prevents any definite conclusion on which neural processes, if any, may be underlying this debilitating symptom. For this reason, the aim of this pilot study was to investigate the functional neural changes associated with cognitive exertion in people with ME/CFS. In particular, due to the consistent observations of structural and functional abnormalities across areas of the SN, i.e. the ACC and the insular cortices, and the lack of clarity regarding the role of alterations in the DMN, the aim of this study was to clarify whether cognitively induced PEM was associated with perturbation of FC in areas within these two large scale brain networks.

2. Methods

2.1. Sample

Twenty participants were recruited for this study between June and October 2013: 10 persons recently diagnosed with ME/CFS, recruited from the Chronic Fatigue Syndrome and Myalgic Encephalomyelitis (ME/CFS) Service - Sheffield Health and Social Care NHS Foundation Trust and 10 age- and education-matched healthy controls without ME/CFS. All participants were women. No strict inclusion criteria were set for participants, apart from meeting the diagnostic criteria for ME/CFS [37]. Ethical approval for this study was granted by the Regional Ethics Committee of Yorkshire and The Humber – Bradford/Leeds (reference number 12/YH/0472). The procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation and with the Declaration of Helsinki.

2.2. Procedure

All participants had their assessment visits in the research facilities at Sheffield Teaching Hospitals NHS Foundation Trust (UK). At the first visit, participants gave written informed consent. Participants then underwent MRI examination lasting approximately 30 min. One participant with ME/CFS could not complete the MRI scan and her data were not included in the final sample. Information on participants' clinical conditions was collected shortly after the MRI assessment: presence of medical conditions (other than ME/CFS), medications, smoking habits (cigarettes per day), drinking habit (alcohol units per week), heart rate (standing and seated) and both systolic and diastolic blood pressure (mmHg, standing and seated). Participants also completed a comprehensive neuropsychological assessment including tests the administration of which does not typically elicit any major cognitive exertion: Verbal Paired Associates Test [38] and Logical Memory Test [38] to evaluate episodic memory, Rey-Osterrieth Complex Figure Test [39] for visuospatial long term memory, Digit Span Test - Forward and Backward [38] and Corsi Block-Tapping Test [40] for verbal and visuospatial working memory, Digit Cancellation Test [41] for attention, the Raven's Advanced Progressive Matrices [42] and the Similarities Test [38] for reasoning and conceptual abilities. Additionally, fatigue and pain were assessed, respectively, by means of the Chalder Fatigue Scale [43] and a Visual Analog Scale (VAS) for Pain, consisting of a 10-cm-long horizontal line with 0 (no pain) at the left extremity and 10 (worst imaginable pain) at the right extremity, on which a participant had to mark the intensity of the pain they were experiencing at that time [44].

Finally, participants completed a series of tasks which are known to trigger significant cognitive exertion. These tasks pose high demands on sustained information processing, sustained and divided attention, working memory, manipulation of mental representations, inhibition of competing information and speed of processing. The tasks selected were

the following: the Stroop Test (3 trials: word reading, colour naming, colour-word inhibition) [45], the Trail Making Test (part A and part B) [46], a modified Category Fluency Task with 3 categories of particular challenge (animals with horns; sports that do not use a ball; things that come in pairs) [47], the Letter Fluency Task (3 letters: P, F and L) [47], the Paced Auditory Serial Addition Test (3 blocks with inter-stimulus intervals of 3 s, 2 s and 1.5 s) [48] and the N-Back Task (1-, 2- and 3-back with letters) [49]. The Paced Auditory Serial Addition Test and the N-Back Task were computer-based and delivered by means of Inquisit version 4.0.3 [50].

Patients with ME/CFS were then instructed to get in contact with the research team as soon as they experienced the onset of PEM, in order to complete a second visit under the influence of PEM. At the second visit, they underwent a second MRI scan and completed the same clinical scales and neuropsychological tests as at baseline. To minimise learning effects parallel versions of sensitive tests were administered.

Six of the 9 persons with ME/CFS experienced PEM and of these, 4 returned for the second visit (1 after 4, 2 after 6 and 1 after 7 days) and completed all retest assessments. The remaining 2 were unavailable for a second visit and as such, no additional data were collected.

The other 3 ME/CFS participants did not report any PEM. Two of these agreed on visiting the clinic to repeat only the effortful cognitive tasks (i.e. those used with the aim to induce PEM), however no subsequent PEM was reported by either participant after 6 and 16 days, respectively, following exertion. They were then invited to return for a scheduled second visit in which they underwent the second scan and the battery of neuropsychological tests (excluding the effortful tasks). The last participant who reported no PEM could not be contacted to attend a second visit and, thus, no follow-up data were collected for this participant. This led to a final sample size of 6 participants with ME/CFS retained for longitudinal statistical analysis (Supplementary file 1, Fig. S1).

All healthy controls returned for a second visit during which they repeated the MRI scan and the same set of clinical scales and neuropsychological tests as the ME/CFS group.

2.3. MRI data acquisition

All MRI data were acquired on a 3 T MR system (Ingenia, Philips Healthcare, Best, The Netherlands) using a 32-channel head-coil with the following parameters:

- 3D sagittal T1-weighted magnetisation-prepared rapid acquisition with gradient echo (MPRAGE) sequence: voxel size = 1.00 × 1.00 × 1.00 mm, slices = 170, TR = 8.2 ms, TE = 3.8 ms, TI = 1000 ms, TFE factor = 222, FOV = 240 × 240 × 170 mm and flip angle = 8°;
- 3D sagittal fluid attenuated inversion recovery (FLAIR) sequence: voxel size = 1.12 × 1.12 × 0.56 mm, slices = 326, TR = 4800 ms, TE = 296 ms, TI = 1650 ms, FOV = 250 × 250 × 182 mm and flip angle = none;
- Two axial resting-state functional, echo planar T2* weighted MRI images with 100 volumes each: voxel size = 0.55 × 0.67 × 4.00 mm, slices = 35, TR = 2600 ms, TE = 35 ms, FOV = 230 × 230 × 140 mm and flip angle = 90°. Thirty seconds of preliminary dummy scans allowed the scanner to reach equilibrium. During MRI acquisitions the participants were asked to remain as still as possible with their eyes closed for the full duration of the scan.

2.4. MRI pre-processing

All MRI data were pre-processed and analysed using SPM12 (Wellcome Centre for Human Neuroimaging, London, UK) running in Matlab R2016b (The Mathworks, Natick, Massachusetts, USA).

T1-weighted and FLAIR images were pre-processed following a procedure previously described in detail [51]. Briefly, T1-weighted images were first reoriented to the bi-commissural plane and segmented into GM, WM and cerebrospinal fluid (CSF). GM maps were then normalised to the standard SPM template in the MNI space and, finally, smoothed using an 8 mm isotropic Gaussian kernel. GM, WM and CSF volumes were extracted using the Matlab function *get_totals*. Similarly, FLAIR images were also reoriented to the bi-commissural plane. WM hyperintensity segmentation was performed by means of the LST toolbox for SPM (www.statisticalmodelling.de/lst.html) [52] combining T1-weighted and FLAIR images. The threshold for automated WM hyperintensities map generation was set at k = 0.3, considered the optimal value to obtain results highly concordant with manual segmentation standards. The total volume of clusters of WM hyperintensities was automatically quantified in millilitres.

Pre-processing of resting-state functional image-sets, instead, was performed using the CONN toolbox [53]. A standard functional pre-processing pipeline was used including the following steps: slice-timing, realignment, detection of outlier volumes by means of the Artifact Detection Tools; co-registration, segmentation into GM, WM and CSF maps and normalisation of functional and structural scans into the MNI space; smoothing using a Gaussian kernel of 8 mm. A series of denoising steps were also carried out: first, aCompCor [54] was used to regress out the first 5 components of WM and CSF signal; second, 12 motion parameters (6 basic parameters and their first derivative) were regressed out [55]; third, scrubbing was performed by removing any invalid volumes [56]; fourth, a band-pass filter (0.008–0.1 Hz) was applied to discard non-neural signals [57]; fifth, linear detrending was performed within each session [53]; finally, despiking was applied in order to reduce the potential weight of any remaining outlier scans after regression [53].

Subsequently, first-level analysis was performed in order to extract, for each participant, the pattern of FC of 3 seeds (selected from the Harvard-Oxford Atlas) widely considered to constitute the main hubs of the SN [17], i.e. the anterior cingulate cortex (ACC), the right and the left insula, and of 4 seeds for the DMN, i.e. the medial prefrontal cortex (MPFC), the posterior cingulate cortex (PCC) and both left and right inferior parietal lobules (IPL). A standard threshold of $r = 0.25$ was used to generate the connectivity maps.

2.5. Statistical analysis

Clinical, cognitive and neuro-volumetric characteristics were compared at baseline in order to detect any between-group differences by using independent-sample *t*-tests and Mann-Whitney *U* tests for normally and non-normally distributed variables, respectively. Differences in handedness were assessed by means of the Chi-squared test. Additionally, voxel-based morphometry analysis was carried out on structural scans to assess regional volumetric GM differences. Repeated measures models with group as a between factor and time as a within factor were used to assess post-exertion changes in the same variables. All analysis were carried out using SPSS Statistics Version 25 (IBM, Chicago, IL, USA) and a significance threshold of $p = 0.05$ was used.

Similarly, the same repeated measures models were also used in SPM12 to investigate post-exertion induced changes in FC in all seeds. Finally, the association between changes found in FC and symptoms was evaluated both in the whole group of participants (both patients and healthy controls) and, as an exploratory analysis, in the ME/CFS sample alone. In order to do so, individual difference maps were created for each seed to obtain maps of FC changes over time (post-exertion - baseline) and comparable difference scores were calculated for the clinical measures that showed significant worsening of symptoms in patients: the Chalder Fatigue Scale and the Pain VAS. Then, SPM12 regression models were created by correlating the difference maps obtained and the difference scores (changes in fatigue and pain) as covariates.

For all MRI analysis performed in SPM12 a significant set threshold of $p = 0.001$ was used, and a family wise error (FWE) correction for multiple comparisons at $p = 0.05$ at cluster level. The coordinates of all

significant results were extracted, converted from the MNI to the Talairach coordinate system and, finally, locations of significant peaks were identified using the Talairach Daemon (http://www.talairach.org/daemon.html).

3. Results

3.1. Clinical profiles

Aside from the number of self-reported medical conditions, people with ME/CFS and healthy controls had similar demographic and biometric profiles and were matched for most of the variables collected (Table 1). Patients had received a diagnosis of ME/CFS shortly prior to the study with onset of symptoms occurring, on average, 26.67 ± 18.95 months before recruitment.

No between-group brain volumetric differences were observed, either in voxel-based or global volume comparisons. Similar clinical profiles were also noted between patients who completed the study and those who dropped out (Supplementary file 1, Table S1).

3.2. Fatigue, pain and cognitive performance

Patients with ME/CFS reported significantly higher baseline levels of pain than healthy controls. A non-significant trend for worse fatigue was also noted in the patient group. Patients also had significantly lower scores on a test of verbal long term memory (Logical Memory Test – delayed recall) (Table 2). Overall, no other differences between the cognitive profiles of the two groups were observed, even on the tests used for cognitive exertion (Supplementary file 1, Table S2).

Similar cognitive profiles were observed between patients with ME/CFS who did and who did not complete the second assessment (Supplementary file 1, Table S3 and S4).

The repeated measures models showed that, after cognitive exertion, patients experienced worsening of both fatigue ($F = 6.53$, $p = 0.023$) and pain ($F = 5.70$, $p = 0.032$) to an extent significantly greater than healthy controls (Table 2). Indeed, fatigue and pain levels reported by healthy controls remained stable over time. On the contrary, no significant change differences on any of the cognitive tests were observed between the two groups. When patients with ME/CFS and healthy controls were pooled, an overall effect of time was observed for some measures: increase in fatigue levels ($F = 4.98$, $p = 0.043$), decreased scores on the Logical Memory Test – immediate recall ($F = 8.38$, $p = 0.012$), and increased performance on the Rey-Osterrieth Complex Figure Test – delayed recall ($F = 10.361$, $p = 0.006$) and on the Similarities Test ($F = 11.49$, $p = 0.004$).

Table 1
Comparison (*t*-test) of baseline demographic and biometric profiles between participant samples (mean and standard deviation), $p < 0.05$.

Characteristic	ME/CFS ($n = 6$)	HC ($n = 10$)	*t*	*p*
Age (years)	42.84 (5.67)	39.30 (8.71)	0.88	0.393
Education (years)	14.50 (3.56)	15.20 (4.24)	−0.34	0.740
Time from symptom onset (months)	26.67 (18.95)	–	–	–
Handedness (R/L)	6/0	9/1	0.64[a]	0.424
Medical conditions (n)	1.50 (8)[b]	0.00 (1)[b]	2.94[c]	0.007
Medications (n)	1.50 (11)[b]	0.00 (2)[b]	1.52[c]	0.181
Smoking (cigarettes per day)	0.00 (8)[b]	0.00 (20)[b]	−0.81[c]	0.692
Drinking (units per week)	0.00 (2)[b]	4.00 (14)[b]	−1.73[c]	0.112
Heart rate - standing	87.00 (45)[b]	71.00 (21)[b]	0.85[c]	0.469
Heart rate - sitting	77.00 (43)[b]	63.00 (26)[b]	1.02[c]	0.371
SBP - standing (mmHg)	122.00 (27)[b]	126.50 (57)[b]	−0.25[c]	0.811
SBP - sitting (mmHg)	114.00 (31)[b]	120.50 (39)[b]	−0.59[c]	0.573
DBP - standing (mmHg)	75.00 (28)[b]	84.50 (36)[b]	−1.01[c]	0.371
DBP - sitting (mmHg)	71.00 (37)[b]	81.00 (29)[b]	−1.02[c]	0.371
GMV (ml)	710.69 (56.79)	701.56 (43.05)	0.37	0.720
WMV (ml)	402.99 (28.89)	383.42 (28.26)	1.33	0.205
WMHV (ml)	0.13 (0.55)[b]	0.14 (11.78)[b]	−0.01[c]	1.000
Time from baseline to retest (days)	6.50 (4)[b]	7.00 (49)[b]	0.11[c]	0.913

DBP: Diastolic blood pressure, GMV: Grey matter volume, SBP: Systolic blood pressure, WMV: White matter volume, WMHV: White matter hyperintensity volume.

[a] Pearson's Chi-squared.
[b] Median (range).
[c] Mann-Whitney *U* test (standardised).

3.3. Functional connectivity

Significant between-group differences emerged from the repeated measures analysis of FC in all 3 SN seeds and in 2 DMN seeds (right IPL and PCC). In particular, patients with ME/CFS had consistently stronger FC than healthy controls in all SN seeds and in the right IPL, but weaker FC in the PCC (Table 3). In the ME/CFS group all 3 SN seeds were more functionally connected with the left superior temporal gyrus and both insulae were more strongly connected with the cerebellar tonsil and the medulla oblongata (Fig. 1). In the DMN, instead, higher FC between the right IPL and both the PCC and the right superior frontal gyrus, but lower FC between the PCC and both the left superior frontal gyrus and the left precentral gyrus were detected in patients.

No effects of time and group-by-time interactions were observed for any of the seeds. However, in order to explore trends of post-exertion changes in FC, analyses of the interaction effects were replicated using a less conservative threshold of $p = 0.05$ at set level (FWE-corrected cluster-level $p = 0.05$). At follow-up, only FC of the right insula was significantly more increased in the ME/CFS group compared to healthy controls. In fact, the right insula appeared to be more strongly connected with a set of areas including the orbito-frontal cortex, the thalamus, the hypothalamus and the basal ganglia (Table 3 and Fig. 1). The nature of cluster significance however, does not permit us to draw inferences about local maxima within the cluster [58].

Since only fatigue and pain levels of patients with ME/CFS were significantly higher than controls after cognitive exertion, we investigated whether such changes in symptoms were associated with increases in the FC of the right insula (Fig. 2 and Table 4). In the whole sample of participants, changes in fatigue were associated with right insula increased FC with the anterior cingulate and decreased connectivity with the cerebellum. In the ME/CFS group, instead, increases in fatigue were negatively associated with decreased connectivity between the right insula and right inferior temporo-occipital areas.

Increases in pain were positively correlated with increases in connectivity between the right insula and the right superior temporal gyrus as well as the left inferior frontal gyrus and the basal ganglia when the sample of patients and healthy participants was analysed jointly. In the ME/CFS group, increased pain was associated with both increased and decreased connectivity with frontal areas.

4. Discussion

This pilot study carried out on two matched groups of women with and without ME/CFS found that patients with ME/CFS had stronger FC than healthy controls between the main hubs of the SN and areas in the left temporal lobe and the medulla. Moreover, connectivity between 2 DMN seeds, i.e. the right IPL and the PCC, and several frontal areas was also found to be altered in the patients compared to the control group: stronger for the right IPL and weaker for the PCC. After inducing cognitive exertion by means of a battery of demanding tests, the ME/CFS group showed increases in self-reported levels of fatigue and pain significantly higher than controls. The right insula was also observed to be more functionally connected to orbito-frontal areas and thalamic and basal ganglia nuclei in patients after exertion. Increases in fatigue and

Table 2
Comparison at baseline (*t*-test) and group-by-time interaction (ME/CFS change vs HC change) effects for fatigue, pain and cognitive performance (mean and standard deviation), $p < 0.05$.

	Baseline				Post-exertion			
Variable	ME/CFS ($n = 6$)	HC ($n = 10$)	*t*	*p*	ME/CFS ($n = 6$)	HC ($n = 10$)	*F*	*p*
Fatigue	21.50 (26)[a]	14.00 (8)[a]	1.19[b]	0.263	36.00 (38)[a]	14.00 (6)[a]	6.53	0.023
Pain	3.05 (7.4)[a]	0.00 (1.4)[a]	2.17[b]	0.007	4.30 (9)[a]	0.00 (1)[a]	5.70	0.032
VPAT	18.00 (3.90)	20.60 (2.41)	−1.66	0.119	16.50 (4.76)	18.30 (5.96)	0.13	0.712
LMT-I	14.67 (4.32)	18.80 (4.34)	−1.85	0.086	13.00 (3.41)	13.90 (4.48)	2.03	0.176
LMT-D	16.67 (4.59)	20.90 (2.47)	−2.42	0.030	17.17 (3.97)	18.70 (2.95)	3.01	0.105
ROCFT-I	34.17 (1.33)	32.90 (2.33)	1.21	0.247	34.33 (1.86)	33.15 (2.33)	0.01	0.939
ROCFT-D	17.50 (6.78)	17.25 (4.76)	0.09	0.932	21.17 (4.80)	22.00 (4.83)	0.17	0.685
DST-F	7.00 (4)[a]	6.00 (2)[a]	0.69[b]	0.562	7.00 (1)[a]	6.00 (3)[a]	<0.01	1.000
DST-B	5.33 (1.37)	5.30 (1.06)	0.05	0.957	5.33 (1.63)	5.50 (1.27)	0.05	0.833
CBTT	5.00 (2)[a]	6.00 (2)[a]	−1.64[b]	0.181	4.50 (2)[a]	6.00 (3)[a]	1.14	0.303
DCT	57.50 (11)[a]	57.00 (8)[a]	0.22[b]	0.875	56.50 (16)[a]	58.00 (6)[a]	0.64	0.436
APM	36.67 (3.88)	40.60 (4.01)	−1.43	0.174	36.83 (2.32)	41.60 (2.59)	1.29	0.275
Similarities	25.00 (5.18)	24.70 (5.01)	0.11	0.910	26.67 (5.09)	26.20 (4.66)	0.03	0.861

APM: Raven's Advanced Progressive Matrices, CBTT: Corsi Block-Tapping Test, DCT: Digit Cancellation Test, DS-F/B: Digit Span Test – forward/backward, LMT-I/D: Logical Memory Test – Immediate/Delayed recall, ROCFT – I/D: Rey-Osterrieth Complex Figure Test – Immediate/Delayed recall, VPAT: Verbal Paired Associates Test.
[a] Median (range).
[b] Mann-Whitney *U* test (standardised).

Table 3
Results (t-statistics) of repeated measures analyses of functional connectivity of the seeds in the salience network and in the default-mode network reported using the Montreal Neurological Institute (MNI) coordinate system, FWE-corrected cluster-level $p < 0.05$.

Seed	Cluster *p*	Cluster extent	Side	Brain region (Brodmann Area)	Peak voxel *p*	*t* value	MNI coordinates		
							x	*y*	*z*
	Salience network - Group effect: ME/CFS > HC								
Anterior cingulate	0.010	181	L	Middle temporal gyrus (BA 21)	< 0.001	5.21	−64	−40	−8
			L	Middle temporal gyrus (BA 21)	< 0.001	4.19	−48	−38	−8
			L	Superior temporal gyrus (BA 22)	< 0.001	3.90	−48	−36	0
Left insula	0.020	166	R	Cerebellar tonsil	< 0.001	5.77	8	−38	−50
			L	Medulla oblongata	< 0.001	3.82	−4	−32	−52
	0.003	240	L	Superior temporal gyrus (BA 22)	< 0.001	5.62	−46	−52	14
			L	Middle temporal gyrus (BA 21)	< 0.001	3.99	−60	−52	2
Right insula	0.001	275	L	Superior temporal gyrus (BA 22)	< 0.001	6.22	−44	−50	14
	0.013	185	R	Cerebellar tonsil	< 0.001	5.57	8	−38	−50
			L	Medulla oblongata	< 0.001	4.60	−4	−32	−54
	Salience network - Interaction effect: $ME/CFS_{POST\text{-}EXERTION}$- $ME/CFS_{BASELINE}$ > $HC_{POST\text{-}EXERTION}$ - $HC_{BASELINE}$								
Right insula	0.044	2062	L	Orbito-frontal cortex (BA 11)	0.001	3.34	−14	28	−10
			R	Thalamus (Ventral lateral nucleus)	0.001	3.31	8	−8	0
			R	Caudate head	0.002	3.18	4	12	−2
			R	Hypothalamus	0.002	3.07	6	−2	−2
			L	Inferior frontal gyrus (BA 47)	0.003	2.96	−20	22	−18
			L	Caudate body	0.003	2.92	−14	12	14
			R	Restrosplenial cortex (BA 26)	0.004	2.88	2	−36	12
			R	Thalamus (Medial dorsal nucleus)	0.004	2.85	2	−6	0
			R	Caudate head	0.004	2.82	12	18	−2
			L	Orbito-frontal cortex (BA 47)	0.004	2.82	−20	26	−28
			L	Orbito-frontal cortex (BA 11)	0.005	2.81	−12	44	−30
			L	Orbito-frontal cortex (BA 11)	0.005	2.78	−14	40	−28
			L	Retrosplenial cortex (BA 26)	0.005	2.74	−2	−36	14
			L	Orbito-frontal cortex (BA 47)	0.006	2.72	−16	26	−30
			R	External globus pallidus	0.006	2.69	10	6	−2
			L	Thalamus (Medial dorsal nucleus)	0.007	2.63	−4	−10	4
	Default-mode network - Group effect: ME/CFS < HC								
Posterior cingulate	0.009	188	L	Precentral gyrus (BA 4)	< 0.001	4.24	−28	−22	72
				Precentral gyrus (BA 6)	< 0.001	4.23	−36	−12	70
				Precentral gyrus (BA 6)	< 0.001	4.19	−38	−26	66
	0.017	166	L	Superior frontal gyrus (BA 10)	< 0.001	6.18	−24	56	20
	Default-mode network - Group effect: ME/CFS > HC								
Right inferior parietal lobule	0.005	225	R	Posterior cingulate (BA 24)	< 0.001	4.54	4	−22	36
			L	Posterior cingulate (BA 23)	< 0.001	4.38	−4	−28	20
			R	Posterior cingulate (BA 23)	< 0.001	4.34	4	−30	28
	0.026	161	R	Superior frontal gyrus (BA 10)	< 0.001	5.33	26	54	−4

pain in the whole sample of participants were correlated, mainly positively, with changes in FC between the right insula seed and frontal and subcortical areas.

From the comparison of baseline characteristics, the two groups appeared to be matched for all demographic, health-related and brain volumetric characteristics. Patients reported higher pain intensity scores

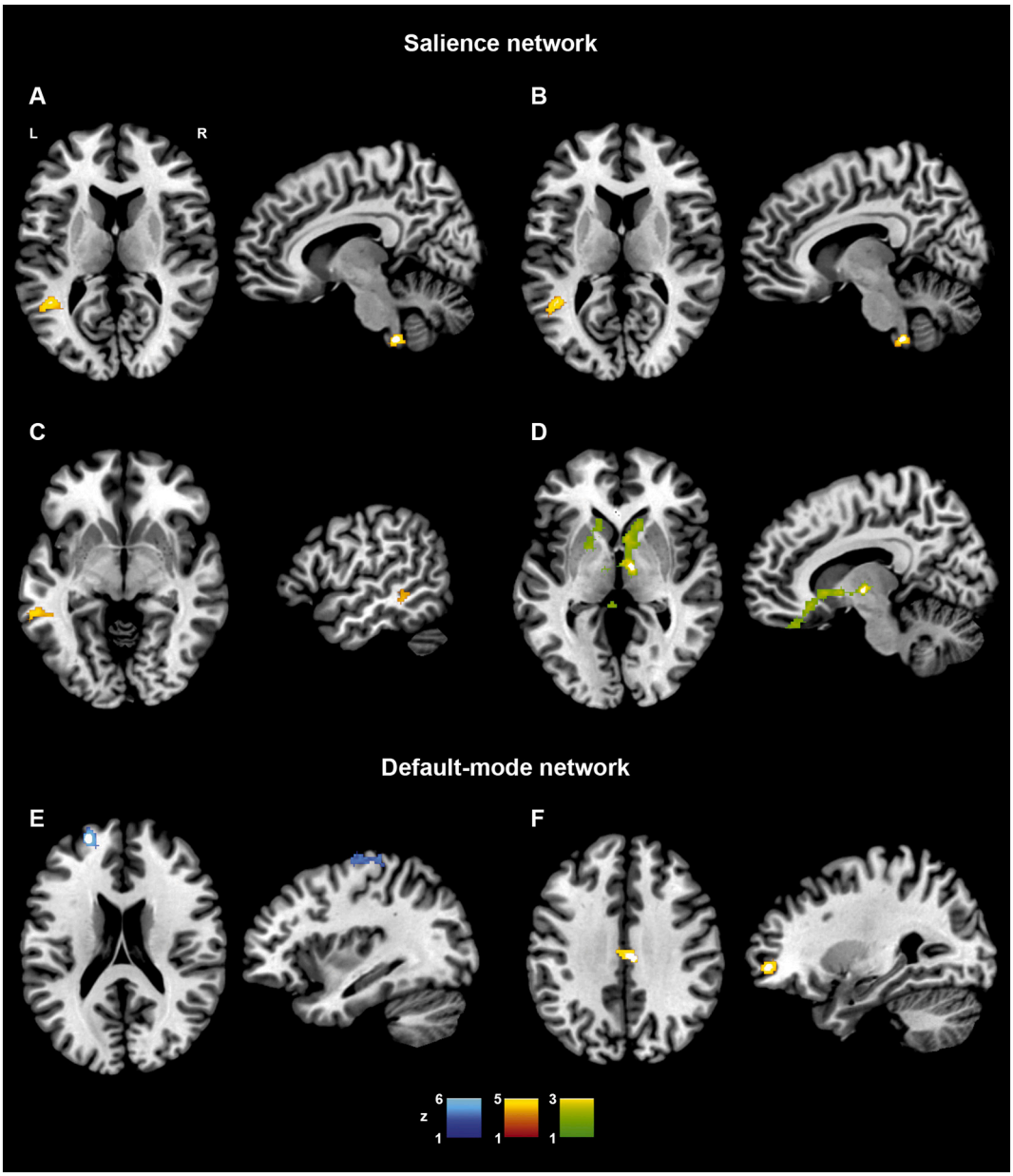

Fig. 1. Results of the repeated measures analyses of functional connectivity of the seeds in the salience network and in the default-mode network (FWE-corrected cluster-level $p = 0.05$). Group effects are highlighted in red (ME/CFS > HC) and blue (ME/CFS < HC) and the group-by-time interaction effect ($ME/CFS_{POST\text{-}EXERTION}$ - $ME/CFS_{BASELINE}$ > $HC_{POST\text{-}EXERTION}$ - $HC_{BASELINE}$) in green for seeds in: A. Left insula; B. Right insula; C. Anterior cingulate cortex; D. Right insula; E. Posterior cingulate cortex; F. Right inferior parietal lobule. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

than controls, while only a trend for a between-group difference in fatigue levels was found, probably because of the small sample size of the patient group that completed the study. However, patients also reported being affected by a higher number of comorbidities additional to ME/CFS, a pattern commonly observed in this clinical population [59,60].

The cognitive profiles of the two groups were also very similar. Significantly, but weakly, worse performance was noted in patients on a task of recall of verbal information. This finding seems to suggest that mild cognitive decline might have occurred in our sample, but to a subclinical extent, in line with current knowledge [1]. The small sample size, however, prevents any clear interpretation of the clinical relevance of such results. Interestingly, after cognitive exertion, no significantly divergent changes in cognition were detected between the two groups. The only time effects observed in the whole sample of participants were decreased performance on immediate recall of verbal information (Logical Memory Test - immediate) and improved performance on tasks of delayed recall of visuospatial information (Rey-Osterrieth Complex Figure Test – delayed) and semantic/logical reasoning (Similarities Test). While these improvements may be due to a practice effect, decline in verbal long-term memory seems to be suggestive of a random effect introduced by the use of alternative versions of the Logical Memory test. However, significant group-by-time interaction effects were found for fatigue and pain measures, with patients experiencing worse symptoms than controls, interpretable as a sign of PEM. Although 2 of the patients who completed the study reported no experience of PEM, even after a second attempt of cognitive exertion, nonetheless a global trend towards worsening of ME/CFS-related symptoms could be clearly detected in the patient sample.

Resting-state fMRI analyses revealed that in patients with ME/CFS all hubs of the SN were more strongly connected with the left Brodmann

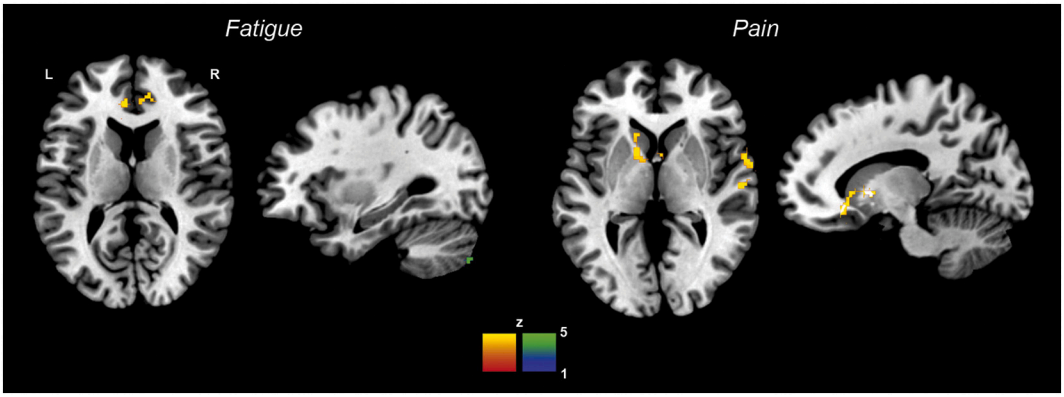

Fig. 2. Significant associations (positive in red and negative in blue) between changes in functional connectivity of the right insula and changes in clinical symptoms in the whole sample of participants, (FWE-corrected cluster-level $p < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 4
Associations (t-statistics) between changes in functional connectivity of the right insula and in fatigue/pain reported using the Montreal Neurological Institute (MNI) coordinate system, FWE- corrected cluster-level $p < 0.05$.

Direction of association	Cluster p	Cluster extent	Side	Brain region (Brodmann Area)	Peak voxel p	t value	MNI coordinates		
							x	y	z
	Fatigue – ME/CFS + Healthy controls								
Positive	< 0.001	306	R	Anterior cingulate cortex (BA 32)	< 0.001	4.46	6	34	22
			L	Anterior cingulate cortex (BA 32)	< 0.001	4.04	−6	40	12
			R	Anterior cingulate cortex (BA 32)	< 0.001	3.72	8	42	10
Negative	0.041	99	L	Inferior semi-lunar lobule	< 0.001	5.25	−44	−74	−50
			L	Pyramis	< 0.001	5.09	−34	−82	−44
			L	Inferior semi-lunar lobule	< 0.001	4.82	−26	−84	−46
	Fatigue – ME/CFS group								
Negative	0.001	56	R	Lingual gyrus (BA 19)	< 0.001	25.10	32	−66	−4
			R	Lingual gyrus (BA 19)	< 0.001	22.93	30	−76	−6
			R	Inferior occipital gyrus (BA 19)	< 0.001	13.48	42	−72	−10
	Pain – ME/CFS + Healthy controls								
Positive	< 0.001	327	R	Superior temporal gyrus (BA 22)	< 0.001	7.96	56	−10	8
			R	Superior temporal gyrus (BA 22)	< 0.001	7.25	66	−8	6
			R	Superior temporal gyrus (BA 22)	< 0.001	6.30	64	4	2
	0.001	181	L	Inferior frontal gyrus (BA 47)	< 0.001	6.20	−12	28	−16
			L	Putamen	< 0.001	5.84	−14	10	2
			L	Caudate head	< 0.001	4.79	−12	20	2
	Pain – ME/CFS group								
Positive	0.006	42	L	Anterior cingulate cortex (BA 24)	< 0.001	30.86	−8	14	30
			L	Anterior cingulate cortex (BA 32)	< 0.001	15.17	−16	22	30
	0.010	39	R	Superior frontal gyrus (BA 9)	< 0.001	17.34	22	28	28
			R	Superior frontal gyrus (BA 9)	< 0.001	12.72	16	32	32
			R	Superior frontal gyrus (BA 9)	< 0.001	10.53	26	32	34
Negative	0.004	44	R	Frontal pole (BA 11)	< 0.001	17.28	18	52	−24
			R	Frontal pole (BA 11)	< 0.001	16.54	12	54	−18

area 22 in the middle and superior temporal gyrus. This area has reportedly been involved in executive processes, i.e. semantic control [61], possibly reflecting a stronger need of salience processing in support of challenging executive tasks for patients with ME/CFS. Moreover, FC between both insulae and the medulla oblongata was also stronger in the ME/CFS group. This finding appears of particular interest, since it may be related to a range of symptoms often observed in this clinical population. The SN is part of a wider network of cortical, subcortical and brainstem areas involved in the central control of autonomic functions [62] as well as pain modulation [63,64]. Greater FC between the insulae and the medulla may suggest an increased interoceptive processing and visceromotor control associated with enhanced experience of fatigue and pain. Such alterations may, in turn, also affect cognitive performance, especially on tasks tapping into a range of attentional and executive functions. In fact, Barnden et al. [25] have observed FC decreases between the medulla, bilaterally, and the left cuneiform nucleus in the midbrain, and between the right medulla and the left subiculum in a sample of patients with CFS while performing the Stroop task.

Additionally, FC alterations were observed also in 2 hubs of the DMN: in the patient group the right IPL was more strongly connected with the PCC and the right superior frontal gyrus, while the PCC was less connected with the left superior frontal gyrus and the left precentral gyrus. These results are partially in line with previous findings [18,19] and suggest that functional brain changes associated with ME/CFS are likely to be multifaceted. Therefore, different ME/CFS symptoms may emerge as a consequence of dysfunction across multiple brain networks.

Group-by-time interaction effects were observed only on the FC pattern of the right insula, when using a less conservative set-level threshold ($p < 0.05$). Interestingly, alterations of the right insula FC have already been reported by previous investigations on ME/CFS [20,21,65] and have been found to be associated with cognitive fatigue

experienced by healthy older adults independently of the cognitive task used [66]. In particular, the right insula appeared to be more strongly connected with the orbito-frontal cortex, involved in reward processing [67], various basal ganglia nuclei, involved in motor, cognitive and motivational functions [68], the ventral lateral and medial dorsal thalamic nuclei, involved in motor [69] and memory functions respectively [70], and the hypothalamus, an important centre for regulation of autonomic functions [62].

The analysis into the associations between changes in the right insula FC and in fatigue and pain measures in the whole sample of participants showed divergent results across symptoms. Indeed, increases in fatigue correlated mainly with increased FC between the right insula and the anterior cingulate cortex and with decreased FC with a small cluster in the cerebellum. Instead, increases in pain intensity correlated with stronger FC between the insula and the right superior temporal gyrus, the inferior frontal/orbitofrontal cortex, the caudate and the lentiform nucleus. These preliminary findings seem to suggest that the observed post-exertion changes in FC of the right insula are mainly in line with the increase of pain intensity experienced by patients with ME/CFS. Therefore, cognitive exertion may induce changes in connectivity between areas assessing internal bodily painful states and those (cortical and subcortical) involved to various degrees in reward representation and executive control functions in line with previous findings [71].

By contrast, fatigue worsening was mainly associated with strengthening of functional coupling between hubs of the SN. This result may be explained by the neurocognitive framework of fatigue proposed by Müller & Apps [72]: the authors suggest that long lasting sensations of fatigue may particularly affect neural systems involved in the cost/benefit evaluation of cognitive efforts, a function performed by areas of the SN. Therefore, cognitive exertion in people with ME/CFS, who already experience high levels of fatigue, may lead to a perturbation in FC between areas that assess internal states and those that support sustained motivation in order to engage with cognitively demanding tasks. The consequence of this process is a worsening of symptoms reported by patients.

Exploratory analyses into the FC-symptom associations in the ME/CFS group only yielded different results from those observed in the whole sample of participants. Fatigue increases were negatively associated with FC changes between the right insula and a right-lateralised cluster in the inferior temporo-occipital cortex, in line with findings by Boissoneault et al. [65] in patients with ME/CFS while performing the Paced Auditory Serial Addition Test. Pain intensity changes, instead, were correlated with FC changes of the right insula with the anterior cingulate cortex and right superior frontal gyrus, positively, and with the right frontal pole, negatively. While these regions are thought to be involved in a wide network of pain-processing areas [63,71], the clinical relevance of such findings still remains elusive, given the very limited sample size of the group of patients who completed the study.

The main limitation of the present pilot study is the small sample size that prevents any definite conclusions on these mainly exploratory findings which, nonetheless, provide insights for further investigations with larger patient groups. Potential effects of cognitive exertion on other brain networks may have gone unnoticed due to lack of statistical power, but also because our analysis was restricted to the hubs of 2 of the main large scale functional brain networks. However, the choice to investigate the SN and the DMN was dictated by our hypothesis that was guided by the findings reported in the available literature on the topic. Additionally, it must be noted that only 4 out of the 6 patients who completed the study experienced PEM. Yet a significant worsening of symptoms, presumably a consequence of cognitive exertion, was detected in patients with ME/CFS compared to controls. Finally, any generalisation of these findings to the whole ME/CFS clinical population is limited by the fact that all recruited participants were women, a choice which was made to minimise sample variability due to gender effects.

5. Conclusions

This pilot study provides preliminary and exploratory findings on the neural consequences of cognitive exertion in women with ME/CFS and their relationship with symptoms. FC of the right insula appears to be particularly perturbed by the cognitive efforts endured by patients and associated with reported increases in fatigue and pain. Strengthening of the coupling between the insula, involved in interoception, and frontal, temporal and subcortical nuclei, involved in reward processing and executive control, was associated with the emergence of PEM. However, given the limitations of this study, only future larger investigations may clarify whether the SN plays a pivotal role in PEM symptoms experienced by the majority of patients with ME/CFS.

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Appendix A. Supplementary data

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