Archival Report

Biological Psychiatry

Prefrontal Structure Varies as a Function of Pain Symptoms in Chronic Fatigue Syndrome

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ABSTRACT

BACKGROUND: Chronic fatigue syndrome (CFS) is characterized by severe fatigue persisting for ≥6 months and leading to considerable impairment in daily functioning. Neuroimaging studies of patients with CFS have revealed alterations in prefrontal brain morphology. However, it remains to be determined whether these alterations are specific for fatigue or whether they relate to other common CFS symptoms (e.g., chronic pain, lower psychomotor speed, and reduced physical activity).

METHODS: We used magnetic resonance imaging to quantify gray matter volume (GMV) and the *N*-acetylaspartate and *N*-acetylaspartylglutamate/creatine ratio (NAA/Cr) in a group of 89 women with CFS. Building on previous reports, we tested whether GMV and NAA/Cr in the dorsolateral prefrontal cortex are associated with fatigue severity, pain, psychomotor speed, and physical activity, while controlling for depressive symptoms. We also considered GMV and NAA/Cr differences between patients with CFS and 26 sex-, age-, and education-matched healthy controls.

RESULTS: The presence of pain symptoms was the main predictor of both GMV and NAA/Cr in the left dorsolateral prefrontal cortex of patients with CFS. More pain was associated with reduced GMVs and NAA/Cr, over and above the effects of fatigue, depressive symptoms, physical activity, and psychomotor speed. In contrast to previous reports and despite a large representative sample, global GMV did not differ between the CFS and healthy control groups.

CONCLUSIONS: CFS, as diagnosed by Centers for Disease Control and Prevention criteria, is not a clinical entity reliably associated with reduced GMV. Individual variation in the presence of pain, rather than fatigue, is associated with neuronal alterations in the dorsolateral prefrontal cortex of patients with CFS.

Keywords: Chronic fatigue syndrome, Dorsolateral prefrontal cortex, Gray matter volume, Magnetic resonance spectroscopy, *N*-acetylaspartate, Voxel-based morphometry

http://dx.doi.org/10.1016/j.biopsych.2016.07.016

Chronic fatigue syndrome (CFS) is characterized by severe fatigue that persists for ≥6 months and leads to considerable impairment in daily functioning. Other criteria include reporting at least four of eight additional symptoms, including pain symptoms and cognitive dysfunction (1,2). The etiology of CFS is unknown; symptoms are not explained by a known medical condition and are not alleviated by rest (3). Our group and others have previously reported reduced gray matter volume (GMV) in patients with CFS compared to healthy controls (HCs) (4–6), an indication that central brain mechanisms might be involved in CFS. However, the clinical and neuronal specificity of cerebral changes in patients with CFS remains unclear.

The cerebral changes observed in patients with CFS might be related to fatigue, but this association has not been shown before. More precisely, it remains to be determined whether reduced GMV is specific to fatigue or whether it relates to other factors that coexist in CFS. Chronic pain, reduced physical activity, and lower psychomotor speed are three factors that are often present in CFS patients and are also known to influence GMV. For instance, chronic pain symptoms in patients with CFS can account for up to one third of impairments in daily functioning (7,8), and CFS is often comorbid with fibromyalgia (9), a condition characterized by chronic widespread pain. Chronic pain conditions, including fibromyalgia, have repeatedly been associated with GMV reductions, especially in prefrontal brain regions (10,11). Physical activity and psychomotor speed are also consistently reported to be reduced in subgroups of patients with CFS (12,13), and both factors have been associated with GMV changes in patients with CFS (4,5). However, physical activity has also repeatedly been associated with (prefrontal) GMV alterations in healthy nonfatigued humans (14,15) and animals (16–18). In sum, fatigue, pain, physical activity, and psychomotor speed contribute to the clinical presentation of CFS, but it is currently unknown whether these factors explain GMV alterations. In addition, despite a clear clinical dissociation between CFS and major depression (19,20), CFS is often associated with increased levels of depressive symptoms (3), which in turn have been associated with reduced GMV, even at subclinical level (21). This study assesses how neuronal structure in patients with CFS is influenced by those five factors.

 ISSN: 0006-3223

We used voxel-based morphometry (VBM) (22) to directly link this study to a number of reports showing CFS-related changes in GMV in the dorsolateral prefrontal cortex (DLPFC) (5,6). VBM has reliably been used to quantify regional GMV alterations in aging (23), neurodegenerative disorders, and various pain disorders (10). However, it remains unclear whether VBM measurements reflect variations in neuronal structure and density, number of glial cells, or variations in vascularization, water content, or interstitial space (17,24). Accordingly, we tested whether GMV changes in DLPFC, as measured with VBM, are driven by neuronal factors, quantifying the metabolite profile of the neurons in that region with magnetic resonance spectroscopy (MRS). We focused on *N*-acetylaspartate (NAA), a metabolite that is found predominantly in neuronal cell bodies and that has shown to be sensitive to neuronal injury (25). As such, NAA has been suggested to provide an in vivo MRS marker for neuronal viability and co-occurrence of GMV and NAA changes in patients with CFS would therefore suggest a neuronal correlate of this disorder.

We collected data from 89 women with CFS and from 26 age-, sex-, and education-matched HCs as part of a randomized controlled trial (26). We tested whether variations in DLPFC GMV and neuronal viability are associated with the defining clinical feature of CFS (i.e., fatigue), or with co-occurring factors (e.g., pain, psychomotor speed, and physical activity) while taking into account the presence of depressive symptoms.

METHODS AND MATERIALS

Participants

Inclusion criteria for all participants were as follows: female, between 18 and 65 years of age,[1] no use of psychotropic medications 6 months before testing (i.e., antidepressants, antianxiety medications, or stimulants), no current psychiatric disorder, except for specific phobias, as assessed with the Mini-International Neuropsychiatric Interview (27), no severe obesity (body mass index $\leq$40 kg/m^2), no contraindication for magnetic resonance examinations, normal hearing and (corrected) vision, and sufficient command of the Dutch language. Additional inclusion criteria for CFS patients were as follows: meeting U.S. Centers for Disease Control and Prevention (CDC) criteria for CFS, including severe fatigue lasting $\geq$6 months and with $\geq$4 additional symptoms (1,2), a score $\geq$40 on the subscale fatigue severity of the checklist individual strength (CIS-fatigue), and a score $\geq$700 on the Sickness Impact Profile 8 (SIP8 total), assessing the level of functional disability.

Consultants of the department of internal medicine evaluated the medical records of referred patients. When the consultants determined that the patients had not been sufficiently examined, they were seen for anamnesis, a full physical examination, a case history evaluation, and laboratory tests following the national CFS guideline, as used at the department of internal medicine, in accordance with the guidelines of the CDC (2,28). Additional inclusion criteria for HCs were a score $<$35 on the CIS-fatigue subscale and no chronic medical condition, including no chronic pain (26). All subjects included in the study provided written informed consent. The study was approved by the local medical research ethics committee (registration number NL43606.091.13) and was conducted according to the principles of the Declaration of Helsinki.

Clinical Assessments

Fatigue severity was measured using the CIS-fatigue subscale, on which scores range from 8 to 56 (29). Physical activity was objectively assessed as the mean activity level during waking hours over a period of 12 days preceding the test sessions using a motion-sensitive actometer worn at the ankle (12). Pain was assessed using diary scores during the 12 days of actometer measurements. Participants were asked to indicate the presence (yes or no) of pain on four time points of the day. Presence of pain was calculated as the percentage of all 48 time points with pain. Following the main additional symptoms of the CDC criteria and previous reports (8), pain was reported for the three most common pain symptoms: muscle pain, joint pain, and headaches. Psychomotor speed was assessed with the digit symbol substitution test of the Dutch Wechsler Adult Intelligence Scale (WAIS-dst) (30). The WAIS-dst was chosen because this measure revealed the strongest correlation with GMV at baseline in our previous study (5). Depressive symptoms were assessed with the Beck Depression Inventory primary care version (BDI-PC) (31). We also report functional disability, as measured with the SIP8 total score (range, 0–5799) (32), physical functioning as assessed with the subscale physical functioning of the Short Form 36 (33), and disease duration in years.

Anatomical Magnetic Resonance Imaging: Image Acquisition and Preprocessing

Magnetic resonance images were obtained on a 3T Siemens Magnetom Skyra magnetic resonance imaging scanner and a 32-channel head coil (Siemens Healthcare, Erlangen, Germany). High-resolution anatomical images were obtained using a T1-weighted magnetization-prepared rapid gradient-echo sequence (repetition time = 2300 ms, echo time = 3.03 ms, flip angle = 8°, 192 sagittal slices, field of view 256 $\times$ 256 mm, voxel size = 1 mm^3, and slice thickness = 1.00 mm). Participants were scanned within a standard time of the day (magnetic resonance imaging scans always began between 10 AM and noon), minimizing the effects of diurnal variations in brain volumes (34).

Images were preprocessed and analyzed using the VBM12 toolbox implemented in the software program Statistical Parametric Mapping (available at www.fil.ion.ucl.ac.uk/spm). VBM is a fully automated technique for computational analysis of differences in global and regional GMVs. Images were first segmented into gray matter, white matter, and cerebrospinal fluid and normalized into standardized anatomical space using the improved Montreal Neurological Institute tissue probability templates provided by SPM12. Images were modulated using global scaling and nonlinear warping to preserve the total amount of GMV. Images were smoothed with a Gaussian kernel of 12 mm full width at half maximum. Global GMV and global white matter volume (WMV) were extracted from native

[1]The initial maximal age of 55 years reported by van der Schaaf *et al.* (26) was extended to 65 years because of the low number of eligible patients.

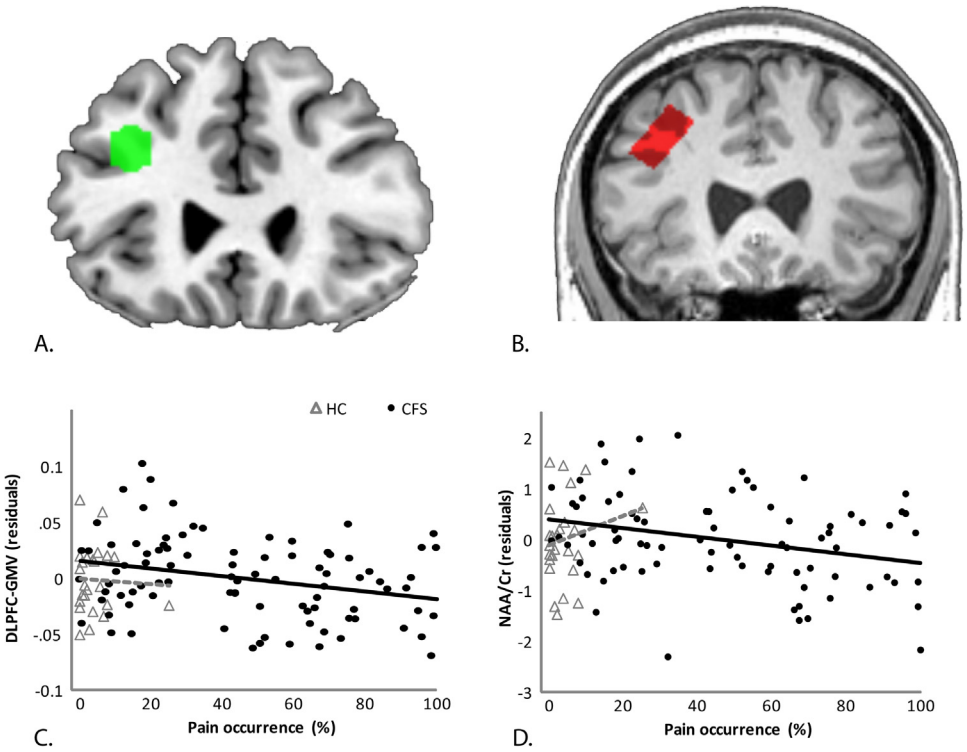

Figure 1. Gray matter volume (GMV) and neuronal metabolism in the dorsolateral prefrontal cortex (DLPFC) of patients with chronic fatigue syndrome (CFS) and healthy controls (HC). **(A)** Region of interest (ROI) used in the analysis of GMV, overlaid on a coronal slide (y = 26) in standardized Montreal Neurological Institute space. The ROI was an 8-mm sphere centered around the between-subject average center of mass of the frontal spectroscopic voxel. **(B)** Representative localization of the frontal spectroscopic voxel, shown on a coronal slice of an individual brain in native space. **(C)** Scatterplot of the relation between GMV and pain occurrence within the ROI of panel **(A)** (DLPFC). The plot describes z-scored GMV corrected for the predictor variables fatigue, activity, and psychomotor speed, and the nuisance variables age, global GMV, and depressive symptoms across patients with CFS (dots) and healthy controls (triangles). **(D)** Scatterplot of the relation between neuronal metabolism (*N*-acetylaspartate and *N*-acetylaspartylglutamate/creatine ratio [NAA/Cr]) and pain occurrence in the DLPFC of patients with CFS and healthy controls. The plot describes z-transformed NAA/Cr corrected for predictor and nuisance variables, as in **(C)**.

space segmented images. Before additional analyses, each T1-weighted scan was manually checked for registration, segmentation, or normalization errors. Finally, statistical inferences are based on two analyses. The first analysis was focused on an a priori volume of interest (VOI), and the second confirmatory analysis explored the whole brain.

VOI. GMV analysis was focused on the left DLPFC region targeted in the MRS scan. Accordingly, we defined a spherical VOI centered on the average center of mass of the MRS voxels across participants (x, y, z = –34, 26, 31), with a radius of 8 mm (Figure 1A). GMV was extracted from this VOI from the smoothed and normalized images of each participant and used for additional analysis (see below).

Whole Brain Regression Analysis. Whole brain regression analyses were conducted to confirm VOI results at the whole brain level. A general linear model was built with pain, psychomotor speed, activity, and fatigue as regressors of interest. Depressive symptoms, age, and global GMV were included as covariates of no interest. We used a whole brain voxel-level statistical threshold of $p < .05$ familywise error corrected (p_{fwe_wb}).

Spectroscopy: Image Acquisition and Preprocessing

Brain metabolite concentrations as reflected by the NAA to creatine ratio (NAA/Cr) were assessed using single voxel proton MRS imaging (repetition time = 1500 ms, echo time = 30 ms, and 64 averages). Water-suppressed MRS spectra were obtained from two voxels (10 × 20 × 30 mm): one voxel was placed in the left middle frontal gyrus (Figure 1B), corresponding to the previous reported region of cognitive behavioral therapy (CBT)–associated gray matter changes in patients with CFS (5). A control voxel was placed in the left calcarine gyrus to assess regional specificity. The scan was acquired using a bandwidth of 200 Hz using 2048 complex datapoints.

Metabolite Analysis. MRS data were analyzed using LCmodel software (version 6.2; available at http://s-provencher.com/pages/lcmodel.shtml). LCmodel is operator-independent software that fits in vivo metabolite spectra using a model resonances basis-set from multiple compounds and phantom solutions acquired during comparable scanner conditions. The basis-set provided by the vendor for high (3T) field strength and short echo time (30 ms) sequence was used for analysis. Contributions of macromolecular and lipid components to the spectra were integrated in this basis set, a method that has shown to improve the fit and to produce reliable NAA/Cr measures (13,14). Because *N*-acetylaspartate and *N*-acetylaspartylglutamate are difficult to dissociate, we used the combined measure of these two metabolites, henceforth referred to as *N*-acetylaspartate. Creatine plus phosphocreatine (Cr) was used as a reference to minimize intra- and interindividual variance. The NAA/Cr ratio was reliably measured with a Cramer-Rao lower bounds <10% in both the DLPFC and V1 voxel, indicating good fit and low metabolite variance. Supplemental Figure S2 shows a representative example of an MR spectrum.

Table 1. Patient Characteristics

	CFS (n = 89)		HCs (n = 26)		CFS vs. HCs
	Mean (SE)	Range	Mean (SE)	Range	t or F Value
Demographics					
Age, years	33.4 (1.2)	(18–60)	32.8 (2.1)	(19–55)	0.248 ns
Education, years (0–7)	4.8 (0.1)	(1–7)	5 (0.2)	(4–7)	–0.593 ns
BMI, kg/m^2	25.1 (0.5)	(17–38)	23.6 (0.8)	(18–35)	1.58 ns
Disease duration, years	6.1 (0.7)	(1–40)			
Functional Status					
CIS-fatigue (8–56)	51.5 (0.5)	(40–56)	16.8 (1.4)	(8–30)	24.179[a]
SIP-total (0–9937)	1718.2 (60.6)	(719–3451)			
SF-36 (0–100)	55.1 (2.4)	(10–100)	94.4 (2.6)	(40–100)	–11.095[a]
BDI-PC (0–14)	3.7 (0.3)	(0–11)	1.08 (0.3)	(0–7)	6.182[a]
Activity Level					
Mean actometer score	66.4 (2.1)	(25–125)	71.8 (3.6)	(27–101)	2.17 ns
Psychomotor Speed					
WAIS-dst (no. correct)	59.4 (1.2)	(30–86)	63.7 (1.8)	(51–85)	3.84 ns
Pain Symptoms					
CDC pain symptoms (0–100)	46 (3.2)	(0–100)	4 (1.0)	(0–25)	6.413[a]

BDI-PC, Beck Depression Inventory for primary care; BMI, body mass index; CDC, U.S. Centers for Disease Control and Prevention; CFS, chronic fatigue syndrome; CIS, Checklist Individual Strength; HCs, healthy controls; ns, not significant; SF-36, Short Form 36 subscale physical functioning; SIP, Sickness Impact Profile; WAIS-dst, Wechsler Adult Intelligence Scale digit substitution.

[a]$p < .001$.

Statistical Analysis. Statistical analyses were performed with SPSS software (version 21; SPSS Inc., Chicago, IL). Between-groups comparisons of demographics, clinical measures, psychomotor speed, activity level, DLPFC GMV, global GMV, and NAA/Cr were performed using two-sided independent sample t tests. Age was added as covariate of no interest in the group comparison of activity level, psychomotor speed, and the brain measures because of known correlations of these measures with age. Global GMV was added as a nuisance regressor in the comparison of DLPFC GMV. Levene's test revealed that the homogeneity of variance assumption was violated for CIS-fatigue, BDI, Short Form 36, and pain. The main group differences were replicated with two subsamples of 26 randomly selected patients (Supplement and Supplemental Table S3).

Two multiple regression analyses (considering either DLPFC GMV or NAA/Cr as dependent variables) were used to assess whether pain, psychomotor speed, activity level, or fatigue severity explained unique sources of interparticipant variance in DLPFC GMV and in NAA/Cr, over and above variance shared across those predictor variables and variance explained by depressive symptoms. Age was added as regressor of no interest in both analyses. Global GMV was added as a nuisance regressor in the analysis of DLPFC GMV. All variables were Z-transformed with a mean of 0 and a standard deviation of 1.

RESULTS

Subjects

Ninety-four patients with CFS and 30 HCs who fulfilled the inclusion criteria were initially included in the study. Because of missing data and acquisition failures, GMV analyses were restricted to 89 patients with CFS and 26 HCs, and MRS analyses were restricted to 81 patients with CFS and 26 HCs. The Supplement includes a full overview on missing data and acquisition failures, and Supplemental Table S2 shows the screening flowchart and full overview of exclusion reasons.

Clinical Characteristics

Following inclusion criteria, CFS patients scored higher than HCs on the CIS-fatigue questionnaire ($t_{113} = 31.23$, $p < .001$). Patients with CFS reported more depressive symptoms (BDI: $t_{113} = 6.18$, $p < .001$), reported more pain symptoms ($t_{113} = 11.54$, $p < .001$), and showed a trend for lower psychomotor speed than HC participants (WAIS-dst: $F_{1,113} = -1.81$, $p = .073$). However, objectively quantified levels of daily physical activity did not differ between the CFS and HC groups ($F_{1,113} = -1.43$, $p = .15$). The two groups were matched on sex, age, and education (age: $t_{113} = .25$, $p = .81$; education: $t_{113} = -.59$, $p = .55$). Other parameters are reported in Table 1.

GMV in DLPFC

Within the CFS patient group, multiple regression analysis with DLPFC GMV as the dependent variable revealed that both presence of pain ($\beta = -.20$, $p = .006$) and physical activity ($\beta = -.16$, $p = .032$) accounted for significant portions of DLPFC GMV variance, while the other clinical measures did not significantly contribute to GMV (fatigue: $\beta = -.11$, $p = .13$; depression: $\beta = .097$, $p = .17$; Figure 1C). More pain was associated with less GMV. In contrast to our previous findings, higher activity level was associated with less GMV in the DLPFC and psychomotor speed was not a significant predictor ($\beta = -.02$, $p = .78$). The overall model explained a considerable amount of variance in the sample ($R^2 = .65$) with pain contributing 8% and activity contributing 5%. The effects

Table 2. Volumes and Metabolite Concentrations

	CFS			HCs			CFS vs. HCs
	Mean (SE)	Range	*n*	Mean (SE)	Range	*n*	*F* Value
VBM							
Global GMV (mL)	671.5 (6.9)	(522–841)	89	672.5 (10.4)	(603–778)	26	0.007 ns
Global WMV (mL)	478.1 (5.2)	(362–624)	89	478.8 (10.2)	(371–553)	26	0.004 ns
DLPFC GMV (mean intensity)	0.330 (0.01)	(0.213–0.495)	89	0.326 (0.01)	(0.254–0.426)	26	0.076 ns
Metabolites							
NAA/Cr DLPFC	1.38 (0.01)	(1.17–1.61)	81	1.37 (0.02)	(1.18–1.6)	26	0.275 ns
NAA/Cr V1	1.56 (0.01)	(1.3–1.81)	81	1.59 (0.02)	(1.34–1.81)	26	2.034 ns

CFS, chronic fatigue syndrome; DLPFC, dorsolateral prefrontal cortex; GMV, gray matter volume; HCs, healthy controls; NAA/Cr, *N*-acetylaspartate/creatine ratio; ns, nonsignificant; VBM, voxel-based morphometry; WMV, white matter volume.

of pain remained significant when removing variance accounted by fatigue, depression, speed, and activity level ($\beta = -.18$, $p = .010$). The effect of activity was only significant when including pain, fatigue, and depression in the model ($\beta = -.16$, $p = .029$) and was nonsignificant without these factors in the model ($\beta = -.087$, $p = .22$).

Within the HC group, none of the variables significantly predicted DLPFC GMV (all $p > .1$). Because pain symptoms did not vary within HCs, subsequent comparison of slopes was only done for activity level. The slopes of the relationships between activity level and DLPFC GMV did not differ between patients with CFS and HCs ($F = 0.75$, $p = .40$).

The spatial specificity of the relationship between pain and DLPFC GMV in the CFS group was confirmed with whole-brain regression analysis, showing a single cluster in the left DLPFC (–33,34,48), corresponding to Brodmann area 9, that was significant at the whole brain level ($T = 4.91$, $k = 1724$, $p_{fwe\_wb} = .029$). No significant clusters were observed for regression analysis with activity, psychomotor speed, or fatigue. No main group difference was found between patients with CFS and HCs on DLPFC GMV ($F_{1,113} = 0.25$, $p = .80$). A median split analysis comparing DLPFC GMV between low pain patients and high pain patients or HCs further confirmed the results from the regression analyses (see Supplement).

NAA/Cr in DLPFC

Within the CFS patient group, multiple regression analysis with DLPFC NAA/Cr as a dependent variable revealed that pain ($\beta = -.28$, $p = .009$; Figure 1D) accounted for significant portions of NAA/Cr variance in the DLPFC. More pain was associated with less NAA/Cr. Activity level ($\beta = -.052$, $p = .64$) did not contribute to NAA/Cr in the DLPFC. Neither psychomotor speed ($\beta = -.17$, $p = .11$), fatigue ($\beta = .13$, $p = .25$), nor depression ($\beta = -.14$, $p = .89$) contributed to NAA/Cr in the DLPFC. The overall model explained a reasonable amount of variance in the sample ($R^2 = .27$), with pain contributing 8%. Within the HC group, none of the variables significantly predicted DLPFC NAA/Cr (all $p > .1$). These effects were specific to the DLPFC. None of the factors predicted NAA/Cr in V1 (pain: $\beta = -.080$, $p = .45$), psychomotor speed ($\beta = -.090$, $p = .48$), activity ($\beta = .011$, $p = .98$), fatigue ($\beta = .042$, $p = .74$), or depression ($\beta = .006$, $p = .96$; overall model fit: $R^2 = .056$). No main group difference was found between patients with CFS and HCs on NAA/Cr (DLPFC: $F_{1,104} = 0.28$, $p = .61$; V1: $F_{1,104} = 2.03$, $p = .16$).

Global GMVs

Pain, activity, or psychomotor speed did not significantly predict global GMV (all $p > .1$). In contrast to previous results, direct comparison between patients with CFS and HCs revealed that the groups did not differ on global GMV ($F_{1,112} = 0.007$, $p = .93$; Table 2), global WMV ($F_{1,112} = 0.004$, $p = .95$), cerebrospinal fluid ($F_{1,112} = 1.361$, $p = .25$), or total intracranial volume (the sum of GMV, WMV, and cerebrospinal fluid: $F_{1,112} = 0.21$, $p = .65$).

A post hoc analysis with Bayesian statistics compared the strength of the evidence provided by the current study and by previously reported samples from our laboratory (4,5) (Supplement). The two patient populations had closely matched clinical profiles (Supplemental Table S1), but the evidence for global GMV changes differed. The current study provides moderate evidence against a global GMV difference between CFS and HC groups (Bayes factor = 0.22). The previous samples provide very strong evidence for a reduction in global GMV in the CFS group (Bayes factor = 36).

Global GMV decreased with age in both groups (CFS: $r = -.54$, $p < .001$; HCs: $r = -.49$, $p = .001$) at an average rate of 3.2 and 2.4 mL per year for patients with CFS and HCs, respectively. This decrease is similar to previously reported age-related GMV decreases in the healthy population (23). Similarly, NAA/Cr also decreased with age in both groups (CFS-middle frontal gyrus: $r = -.406$, $p < .001$; CFS-V1: $r = -.208$, $p = .062$; HC-middle frontal gyrus: $r = -.49$, $p = .011$; HC-V1: $r = -.52$, $p = .007$).

DISCUSSION

This study assessed whether gray matter reductions in the DLPFC of CFS patients is associated with fatigue or with other co-occurring symptoms (e.g., pain, activity, and psychomotor speed), whether these reductions were of neuronal origin, and whether (global) GMV was reduced compared to healthy controls as reported previously (4–6). VBM and NAA/Cr were used to index GMV and neuronal viability in the DLPFC, respectively.

Pain, Rather Than Fatigue, Modulates GMV and NAA/Cr in the DLPFC

Both GMV and NAA/Cr in the DLPFC decreased as pain symptoms occurrence increased, whereas fatigue and other

co-occurring symptoms were not related to GMV and NAA/Cr in the DLPFC of patients with CFS. These effects were regionally specific, as confirmed with whole brain regression analysis and assessment of NAA/Cr in a control region (V1). The concomitant reductions of GMV and NAA with increasing pain suggest that the GMV effects reflect pain-related alterations in neuronal viability or functioning (25,35). These findings suggest that variations in GMV in the DLPFC of patients with CFS is robustly predicted by the presence of pain, a symptom that is part of the additional symptom list of the CDC criteria (8,9) and are in line with multiple reports of prefrontal GMV reductions in chronic pain conditions (10,11,36–38), as well as with reports of concomitant reductions of GMV and NAA in chronic pain (25,35,39). Retrospectively, these findings also fit with results of a previous study on fatigued cancer survivors whose GMV fell within the range of healthy controls (40). Those patients are severely fatigued but generally do not have additional pain symptoms (41). Accordingly, it is possible that our results reflect brain alterations linked to chronic pain rather than fatigue.

Anatomically, the DLPFC has direct access to limbic structures, such as the anterior cingulate cortex, basal ganglia, and amygdala/hippocampal complex, as well as to somatosensory and motor regions. This connectivity profile could support modulatory effects of expectations and reappraisal on pain-related afferences (42). Accordingly, the DLPFC has consistently been involved in controlling expectancy effects on pain (43,44) and the top-down modulation of pain-related regions (45). The neuronal modifications observed in patients with CFS with increased occurrence of pain could therefore reflect a reduced ability of the DLPFC to control nociceptive processing. This interpretation fits with known morphologic alterations in the DLPFC of patients with chronic pain after various interventions (46–49). For instance, CBT in patients with chronic pain has led to increases in DLPFC GMV (47). Similarly, CBT in CFS patients led to increased DLPFC GMV (5). These observations suggest that increased DLPFC-GMV may be an index of compensatory mechanisms triggered by effective CBT, likely involving descending pain modulation. Indeed, during CBT for CFS, both fatigue and pain decrease, as well as the contribution of cognitive factors, such as catastrophizing (8,50–52). However, these studies also revealed that cognitive-behavioral factors that predict fatigue and pain reductions after CBT only partly overlap, suggesting different underlying mechanisms. The completion of the randomized clinical trial in which this study is embedded will allow us to empirically test whether alterations in DLPFC GMV are associated with reductions in pain or fatigue (26).

Comparison With Previous Cohorts

We expected to replicate a reduction in global GMV previously observed in patients with CFS (4,5). In fact, the current and the previous datasets provide contradictory findings. Bayesian inference indicates that there is strong evidence for a reduction in global GMV in the previous dataset, but moderate evidence against that difference in the current dataset. Other studies on structural cerebral alterations in CFS have also reported inconsistent findings. Some studies report different regional GMV reductions (6,53), and others report no GMV differences (54). These repeated GMV inconsistencies have been obtained in the context of comparable clinical profiles of CFS across studies. One possible explanation is that GMV is associated not with fatigue (or CFS diagnosis) per se, but rather with factors that contribute with varying extent to the clinical presentation of CFS. The current study is the first to be adequately powered for looking into these within-group variations. It indicates that pain might account for variable GMV results in CFS literature. Future studies on structural correlates of CFS will need to pay attention to those co-occurring factors, including presence of pain symptoms, that are often present only in subgroups of patients with CFS (8,9,12,13).

Interpretational Issues

It might be argued that the current findings and the disparity with the findings of de Lange *et al.* (4,5) are caused by a recruitment bias in the CFS sample. In fact, our supplementary analysis revealed that both patient populations showed similar differences in fatigue severity and functional disability from the HCs. Moreover, formal statistical comparison of the main clinical characteristics in the current and previous samples (5,55) did not identify significant differences between the patient groups with respect to their demographics, functional status, disease duration, activity level, or psychomotor speed, and revealed even moderately higher occurrence of pain in the current sample. Similarly, there were no statistical differences between the control groups (Supplement). However, it is possible that the current CFS sample might have had a lower occurrence of psychiatric comorbidities, given that we used structured interviews by trained cognitive behavioral therapists, which were not yet common practice at our center at the time of the previous studies (4,5).

In contrast to the findings of many cross-sectional and intervention studies on the effects of physical exercise on prefrontal GMV (15,56), we found a marginally negative or absent relationship between physical activity and GMV or NAA/Cr, respectively. In fact, recent insights suggest that exercise-related morphological changes may critically depend on the type of exercise. GMV increases are only seen when the exercise involves environmental enrichment or coordinative training (i.e., balance or hand–eye coordination), while aerobic or cardiorespiratory exercise alone does not necessarily lead to GMV increases (17,56,57). In this study, physical activity was quantified with an actometer, and this index might not capture environmental enrichment or coordinative training. A second possibility is that other factors considered in the multiple regression model might have captured variance related to environmental enrichment.

In line with other reports (8,9) we based the pain measure on self-reports of the presence of pain rather than the severity of pain. It is likely that patients reporting more pain may also have used more pain medications. Although occasional use of over-the-counter paracetamol and nonsteroidal anti-inflammatory drugs were allowed, it is unlikely that pain medication explains the pain-related GMV reductions, because nonsteroidal antiinflammatory drugs have been shown to prevent, rather than increase, declines in GMV (58,59).

Finally, in addition to the CDC criteria, several other case definitions for CFS exist. Two of those are the Canadian criteria (60) and the newly established Institute of Medicine criteria (61). The major difference between these criteria is

the evaluation of additional symptoms (i.e., besides severe fatigue). Accordingly, it could be argued that inconsistencies in the literature may relate to the criteria used. However, most studies investigated GMV in CFS using CDC criteria (4–6,53), and only one study used the Canadian criteria (54). Using other criteria is unlikely to resolve inconsistencies in the literature. First, symptoms considered as compulsory in the Institute of Medicine criteria (unrefreshing sleep) or Canadian criteria (postexertional malaise and pain symptoms) were present in >80% of the patients tested in the current study (Supplemental Table S4), suggesting large overlap between the case definitions. Second, many symptoms remain optional or can vary considerably with respect to severity or frequency and could therefore still account for large variance within the patient group.

Conclusions

We present anatomical data from the largest cohort of patients with CFS assembled so far ($N = 89$) showing that the presence of pain symptoms is an important predictor for both GMV and NAA/Cr in the DLPFC. In contrast, the main clinical characteristic of CFS, fatigue severity, did not contribute to GMV. Given similar GMV alterations in other chronic pain conditions without fatigue, these results show that reduced GMV is not specific to the clinical entity CFS, as defined by the CDC criteria.

ACKNOWLEDGMENTS AND DISCLOSURES

This work was funded by a private foundation that wishes to stay anonymous. The identity of the foundation was disclosed to the editors and does not constitute, to the *Journal's* understanding, a conflict of interest. The funder had no role in the study design, data collection, data analysis, decision to publish, or preparation of the manuscript.

All listed authors concur in submission and the final manuscript has been approved by all. Experimental procedures have been conducted in conformance with the policies and principles contained in the Federal Policy for the Protection of Human Subjects and in the Declaration of Helsinki.

All authors report no biomedical financial interests or potential conflicts of interest.

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Received Mar 24, 2016; revised July 22, 2016; accepted July 26, 2016.

Supplementary material cited in this article is available online at http://dx.doi.org/10.1016/j.biopsych.2016.07.016.

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