

# Volumetric differences in hippocampal subfields and associations with clinical measures in myalgic encephalomyelitis/chronic fatigue syndrome

Kiran Thapaliya<sup>1,2</sup>  | Donald Staines<sup>1</sup> | Sonya Marshall-Gradisnik<sup>1</sup> | Jiasheng Su<sup>1</sup> | Leighton Barnden<sup>1</sup> 

<sup>1</sup>National Centre for Neuroimmunology and Emerging Diseases, Menzies Health Institute Queensland, Griffith University, Gold Coast, QLD, Australia

<sup>2</sup>Centre for Advanced Imaging, The University of Queensland, Brisbane, QLD, Australia

## Correspondence

Kiran Thapaliya, National Centre for Neuroimmunology and Emerging Diseases, Mailbox 68, Menzies Health Institute Queensland, Building G40, Level 9, Griffith University, Parklands Drive, Gold Coast, QLD 4222, Australia. Email: [k.thapaliya@griffith.edu.au](mailto:k.thapaliya@griffith.edu.au)

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## Abstract

Myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) patients suffer from a cognitive and memory dysfunction. Because the hippocampus plays a key role in both cognition and memory, we tested for volumetric differences in the subfields of the hippocampus in ME/CFS. We estimated hippocampal subfield volumes for 25 ME/CFS patients who met Fukuda criteria only (ME/CFS<sub>Fukuda</sub>), 18 ME/CFS patients who met the stricter ICC criteria (ME/CFS<sub>ICC</sub>), and 25 healthy controls (HC). Group comparisons with HC detected extensive differences in subfield volumes in ME/CFS<sub>ICC</sub> but not in ME/CFS<sub>Fukuda</sub>. ME/CFS<sub>ICC</sub> patients had significantly *larger* volume in the left subiculum head ( $p < 0.001$ ), left presubiculum head ( $p = 0.0020$ ), and left fimbria ( $p = 0.004$ ). Correlations of hippocampus subfield volumes with clinical measures were stronger in ME/CFS<sub>ICC</sub> than in ME/CFS<sub>Fukuda</sub> patients. In ME/CFS<sub>Fukuda</sub> patients, we detected positive correlations between fatigue and hippocampus subfield volumes and a negative correlation between sleep disturbance score and the right CA1 body volume. In ME/CFS<sub>ICC</sub> patients, we detected a strong negative relationship between fatigue and left hippocampus tail volume. Strong negative relationships were also detected between pain and SF36 physical scores and two hippocampal subfield volumes (left: GC-ML-DG head and CA4 head). Our study demonstrated that volumetric differences in hippocampal subfields have strong statistical inference for patients meeting the ME/CFS<sub>ICC</sub> case definition and confirms hippocampal involvement in the cognitive and memory problems of ME/CFS<sub>ICC</sub> patients.

## KEYWORDS

Fukuda, hippocampus, International Consensus Criteria, memory, myalgic encephalomyelitis/chronic fatigue syndrome

## 1 | INTRODUCTION

Myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) is a complex illness characterized by a range of symptoms that

includes fatigue, malaise, headaches, sleep disturbances, difficulties with concentration and cognitive function, and muscle pain (Baker & Shaw, 2007). The cognitive symptoms include deficits in memory, attention, reaction time, information processing speed, and

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free memory recall (Cockshell & Mathias, 2010). The diagnosis of ME/CFS has evolved from symptoms that meet the Fukuda criteria (Fukuda, 1994), Canadian Consensus Criteria (CCC) (Carruthers et al., 2003), and International Consensus Criteria (ICC) (Carruthers et al., 2011). The Fukuda (Fukuda, 1994) definition requires patients to meet fatigue severity criteria and exhibit four of eight other symptoms but does not include specific neurocognitive, cardiorespiratory, or thermoregulatory impairments. Criteria were refined to include autonomic and flu-like symptoms in CCC and further revised to include neurocognitive, cardiorespiratory, and thermoregulatory impairments in ICC (Carruthers et al., 2011). Hence the ICC is more selective for ME/CFS patients than the Fukuda definition.

ME/CFS patients experience memory and cognitive deficit (Robinson et al., 2019).

The hippocampus is an extension of the temporal lobe of the cerebral cortex (Gilbert & Brushfield, 2009) and plays an important role in cognitive functions such as memory, executive processing, and reward processing (Brown et al., 2013). The hippocampus is a complex anatomical structure consisting of major subfields (dentate gyrus, subiculum, parasubiculum, entorhinal cortex, and the three cornu ammonis [CA1, CA3, CA4]), each with distinct memory function. The subfields of the hippocampus may be involved in selective neurocognitive processes in health (Middlebrooks et al., 2017) and diseases (Small, 2014). Hippocampal volume reduction has been reported in Alzheimer's disease (Dhikav & Anand, 2011), depression and schizophrenia (Bast, 2011), epilepsy (Lv et al., 2014; Schoene-Bake et al., 2014), hypertension (Dhikav & Anand, 2007), and Cushing's disease (Santos et al., 2014). Alterations of hippocampus subfields have also been reported in neurodegenerative diseases (Anand & Dhikav, 2012). One or more of the CA1, CA3, CA4, subiculum, parasubiculum, presubiculum, HATA, or fimbria had reduced volume in mild cognitive impairment, Alzheimer's disease, post-traumatic stress disorder, medial temporal lobe epilepsy, hippocampal sclerosis, schizophrenia, bipolar disorder, and alcoholic use disorder patients (Aas et al., 2014; Bøen et al., 2014; Braak & Braak, 1997; Carlesimo et al., 2015; Chen et al., 2018; Györfi et al., 2017; Hanseeuw et al., 2011; Haukvik et al., 2015; Hayes et al., 2017; Janiri et al., 2019; Khan et al., 2015; La Joie et al., 2013; Mak et al., 2016; Mathew et al., 2014; Postel et al., 2019; Roddy et al., 2019; Shim et al., 2017; Zahr et al., 2019). Neuronal loss in the CA1 and subiculum was reported in Alzheimer's disease (Rössler et al., 2002; Schönheit et al., 2004).

Saury (Saury, 2016) described the role of the hippocampus in neurocognitive deficits, disturbance in the regulation of stress response, and pain perception in ME/CFS. It has been reported that 89% of ME/CFS patients have memory and concentration problems, and difficulties in processing complex information (Jason et al., 2012). In studies involving neuropsychological tests of attention, working memory, and processing speed, ME/CFS patients performed significantly worse than HC (Marcel et al., 1996; Vercoulen et al., 1998).

No previous study has investigated volumetric differences in the subfields of the hippocampus in ME/CFS patients relative to HC.

### Significance

Our study found left hippocampal subiculum, presubiculum, and fimbria volumes were significantly larger in ME/CFS<sub>ICC</sub> patients compared with HC, but not for ME/CFS<sub>Fukuda</sub> patients. Furthermore, this study demonstrated that multiple hippocampal subfield volumes are different in ME/CFS<sub>ICC</sub> patients meeting the strict ICC case definition, and they exhibited strong associations with clinical measures. Therefore, the strict case definitions are essential in investigation of the pathophysiology of ME/CFS. Subiculum and parasubiculum volumes were larger in ME/CFS in contrast to reductions seen in other neurological disorders.

Because the Fukuda and ICC classifications differ substantially, we avoided increased variance in a combined group by studying each group separately. Therefore, the specific aims of this preliminary study were a) to estimate the subfield volumes of the hippocampus in ME/CFS patients and compare with HC, and b) to investigate the relationship between hippocampal subfield volumes and clinical symptom severity of ME/CFS patients.

## 2 | MATERIALS AND METHODS

### 2.1 | Participant recruitment

The study was approved by the local human ethics (HREC/15/QGC/63 and GU:2014/838) committee of Griffith University and the Gold Coast University Hospital where scanning was performed. Written informed consent was obtained from all individuals. Twenty-five ME/CFS patients meeting only the Fukuda criteria (Fukuda, 1994), 18 ME/CFS patients meeting the ICC criteria (Carruthers et al., 2011), and 25 age-matched healthy controls were recruited (see Table 1 for demographic information) through an online Lime survey. Furthermore, HC and ME/CFS patients were excluded from this study if they had hyper/hypotension, autoimmune disease, or were pregnant or breastfeeding.

### 2.2 | Clinical measures

Clinical measures from ME/CFS patients were collected as follows. The 36-item SF36 short-form health survey questionnaire (Alonso et al., 1995), was completed by all participants and "pain," "fatigue," and SF36 Physical (phys\_all) scores were extracted. An information processing score (procinfo) and a sleep disturbance score (SDS) were obtained via a survey: "In the past month, how severe were the following symptoms (on a scale of 1 to 10, 1 being not a problem, 10 being extremely severe)" for symptoms "Difficulty processing information?" and "Sleep disturbances?"

TABLE 1 Demographic and clinical characteristics of patients with ME/CFS and HC

	ME/CFS <sub>Fukuda</sub> (n = 25)	ME/CFS <sub>ICC</sub> (n = 18)	HC (n = 25)	p Value
Age	49.8 ± 12.2	43.2 ± 10.7	43.1 ± 13.7	0.1 <sup>a</sup> , 0.8 <sup>b</sup>
M/F	5/20	6/12	9/17	N/A
Pain	43.1 ± 21.5	38.5 ± 23.3	92.0 ± 10.0	<0.001 <sup>a,b</sup>
phys_all	29.2 ± 13.3	28.3 ± 14.2	83.2 ± 18.1	<0.001 <sup>a,b</sup>
Fatigue	18.4 ± 17.0	4.0 ± 4.9	71.3 ± 17.7	0.92 <sup>a</sup> , 0.96 <sup>b</sup>
SDS	6.5 ± 1.8	7.0 ± 1.9	1.9 ± 1.5	<0.001 <sup>a,b</sup>
Procinfo	5.8 ± 1.8	6.7 ± 1.3	1.5 ± 1.0	<0.001 <sup>a,b</sup>

Note: Superscript a and b are the p values for ME/CFS<sub>Fukuda</sub> versus HC and ME/CFS<sub>ICC</sub> versus HC, respectively.

Abbreviations: ME/CFS, myalgic encephalomyelitis/chronic fatigue syndrome; M/F, male/female; phys\_all, SF36 physical score; Procinfo, information processing score; SDS, sleep disturbance score.

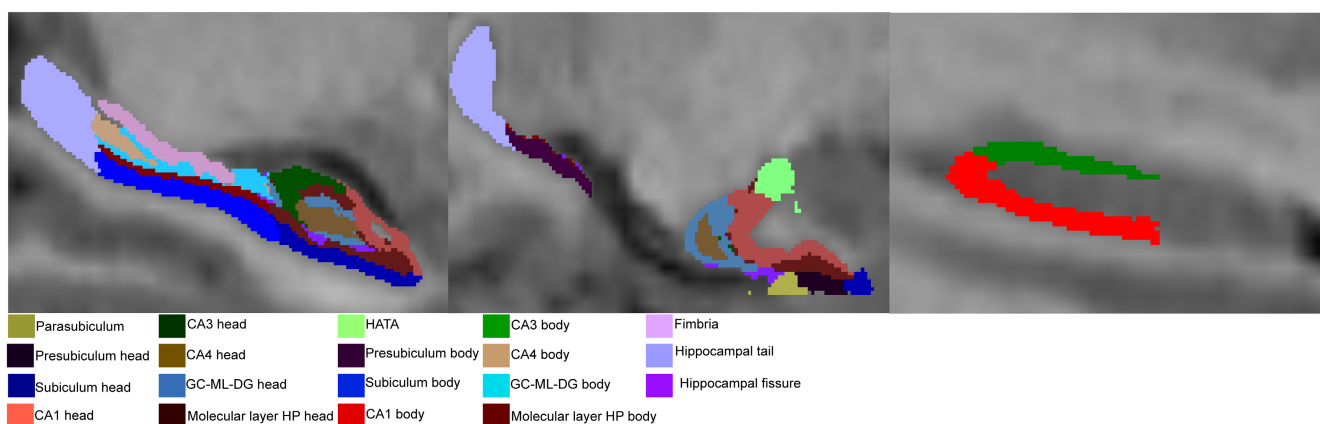


FIGURE 1 Visualizations of hippocampal subfields of a healthy participant. Subfields are color coded. CA, cornu ammonis; GC-ML-DG, granule cell layers of the dentate gyrus; HATA, hippocampus-amygdala transition area

### 2.3 | MRI scans and data processing

The T1-weighted data were acquired using a 3T Skyra MRI scanner (Siemens Healthcare, Erlangen, Germany) with a 64-channel head-neck coil (Nova Medical, Wilmington, USA). Three-dimensional anatomical images were acquired using a T1-weighted magnetization prepared rapid gradient-echo (MPRAGE) sequence with a repetition time (TR) = 2,400ms, echo time (TE) = 1.81ms, flip angle = 8°, acquisition matrix = 224 × 224 × 208, and voxel size 1mm × 1mm × 1mm. The total acquisition time for T1w MPRAGE scans was 8:20min:sec. MR images were acquired in both patients and HC with the same scanner, using the same scanning parameters.

T1 MPRAGE images were anatomically segmented using FreeSurfer version 7.1.1 (Fischl, 2012) (<https://surfer.nmr.mgh.harvard.edu/>) using the default FreeSurfer command “recon-all” on a Macintosh computer (Operating system: Catalina, RAM = 36GB, and core: 8). The “recon-all” processing includes motion correction, nonlinear spatial normalization to Talairach space, intensity normalization, removal of non-brain tissue, cortical parcellation, subcortical segmentation, gray and white matter boundary tessellation,

automated topology correction, and surface deformation. Detailed information about the pipeline can be found at (<https://surfer.nmr.mgh.harvard.edu/fswiki/recon-all>). The intracranial volume of each participant was calculated.

Hippocampus subfield segmentation was performed via the Free-surfer 7.1.1 hippocampus subfield module (Iglesias et al., 2015) as shown in Figure 1. Using this module, the left and right hippocampal subfields: head, body, and tail; parasubiculum, presubiculum, subiculum, cornus ammonis (CA1, CA3, and CA4) head and body; granule cell layers of the dentate gyrus (GC-ML-DG), molecular layer of the hippocampus, fissure, fimbria, and hippocampus-amygdala transition area (HATA) were defined. All hippocampal subfields were visually checked for distortion-free segmentation. Two ME/CFS<sub>ICC</sub> patients and one ME/CFS<sub>Fukuda</sub> patients were excluded from analysis due to inadequate segmentation.

### 2.4 | Statistical analysis

Univariate general linear model (GLM) statistical analysis was performed to test hippocampal subfield volume differences between

ME/CFS patients and HC using SPSS version 27. After confirmation of homogeneity using Levene's test, the univariate GLM was used to test for group differences. Correction for multiple comparisons was implemented using false discovery rate (FDR). Then Spearman's correlations were performed between hippocampal subfield volumes and clinical severity measures for ME/CFS<sub>Fukuda</sub> and ME/CFS<sub>ICC</sub> defined patients. The normality condition for data was checked using the Shapiro-Wilk method available in SPSS before the correlation. Age, sex, and intracranial volume (ICV) were used as covariates for group comparison and correlation analysis.

### 3 | RESULTS

#### 3.1 | Group comparison: ME/CFS<sub>Fukuda</sub> versus HC

When we compared hippocampal subfield volumes between ME/CFS<sub>Fukuda</sub> and HC, most subfield volumes were smaller in ME/CFS<sub>Fukuda</sub> patients. The right CA1 body volume difference was statistically significant ( $F(1,44) = 6.49; p = 0.014$ ) only before the multiple comparison correction (see Table 2). All subfield differences are listed in Table S1).

#### 3.2 | Group comparison: ME/CFS<sub>ICC</sub> versus HC

The comparison of ME/CFS<sub>ICC</sub> and HC hippocampal subfield volumes detected three subfield volumes that were significantly smaller in ME/CFS<sub>ICC</sub> patients: the left CA1 body ( $F(1,36) = 7.32; p = 0.01$ ), CA3 head ( $F(1,36) = 6.84; p = 0.013$ ), and CA3 body ( $F(1,36) = 5.40; p = 0.026$ )

and larger in the right subiculum head ( $F(1,36) = 6.41; p = 0.016$ ) only before the multiple comparison correction (see Table 2). We only observed significantly larger volumes in three subfields: the left subiculum head (left:  $F(1,36) = 19.15; p < 0.001$ ), left presubiculum head ( $F(1,36) = 11.565; p = 0.002$ ), and left fimbria ( $F(1,36) = 9.44; p = 0.004$ ) (see Table 2) which survived the multiple comparison correction. Differences for all subfields are listed in Table S2.

#### 3.3 | Hippocampal subfield volume correlations with clinical measures

Correlations were performed between subfield volumes and five clinical measures: fatigue, pain, phys\_all, procinfo and SDS, controlling for age, sex, and ICV after the normality test. This was implemented separately for the ME/CFS<sub>Fukuda</sub> and ME/CFS<sub>ICC</sub> cohorts.

##### 3.3.1 | ME/CFS<sub>Fukuda</sub>

For ME/CFS<sub>Fukuda</sub> patients, we observed statistically significant positive correlations (see Table 3) between "fatigue" and volume of the left hippocampal tail ( $r(19) = 0.465, N = 24, p = 0.039$ ), left parasubiculum ( $68.44 \pm 11.40; r(19) = 0.564, N = 24, p = 0.01$ ), left HATA ( $63.43 \pm 10.77; r(19) = 0.513, N = 24, p = 0.021$ ), left whole hippocampal head ( $1,744.18 \pm 205.94; r(19) = 0.47, N = 24, p = 0.037$ ), and left whole hippocampus ( $3,583.20 \pm 370.24; r(19) = 0.478, N = 24, p = 0.033$ ). A positive correlation implies more severe fatigue was associated with larger volumes. We also observed moderate negative relationships between "SDS" and right CA1 body volume

TABLE 2 The mean and standard hippocampal subfield volumes of ME/CFS<sub>ICC</sub> or ME/CFS<sub>Fukuda</sub> patients statistically different to HC ( $p < 0.05$ )

	Volume in mm <sup>3</sup>		p Value	95% confidence interval		Effect size
				Lower	Upper	
<i>Left hippocampus</i>						
	ME/CFS <sub>ICC</sub>	HC				
CA1 body	126.4 ± 17.4 ↓	143.25 ± 21.7	0.01	-31.4	-4.5	0.169
CA3 head	128.4 ± 11.0 ↓	138.9 ± 14.3	0.013	-17.3	-2.2	0.160
CA3 body	85.43 ± 16.01 ↓	97.90 ± 15.37	0.026	-22.8	-1.5	0.131
Subiculum head	204.0 ± 18.1 ↑	187.7 ± 13.8	<0.001**	13.8	37.7	0.348
Presubiculum head	151.3 ± 13.3 ↑	145.0 ± 9.7	0.002**	4.7	18.8	0.243
Fimbria	86.5 ± 21.9 ↑	72.0 ± 13.5	0.004**	5.7	28.0	0.208
<i>Right hippocampus</i>						
Subiculum head	204.7 ± 19.5 ↑	190.8 ± 13.2	0.016	3.0	27.7	0.151
	ME/CFS <sub>Fukuda</sub>	HC				
CA1 body	139.3 ± 18.6 ↓	156.8 ± 23.6	0.014	-30.4	-3.5	0.129

Note: ↓ indicates a smaller volume in ME/CFS patients than HC and ↑ indicates a larger volume in ME/CFS than HC. The effect size was determined by partial eta squared ( $\eta^2$ ).

\*\*Represents statistically significant after adjusting for multiple comparison. Univariate GLM statistical analysis was performed between two groups using SPSS software version 27.

TABLE 3 Correlation between hippocampal subfield volumes and clinical measures in ME/CFS<sub>Fukuda</sub>

Hippocampal subfield	Clinical measures	r	p	df
Left hippocampal tail	fatigue	0.465	0.039	19
Left parasubiculum		0.564	0.01	19
Left HATA		0.513	0.021	19
Left whole hippocampus head		0.47	0.037	19
Left whole hippocampus		0.478	0.033	19
Right CA1 head		0.518	0.019	19
Right molecular layer HP head		0.529	0.016	19
Right CA1 body	SDS	-4.87	0.025	19

Note: The Spearman correlation test was used to perform correlation analysis using SPSS software version 27.

Abbreviations: df, degrees of freedom; r, correlation coefficient; SDS, sleep disturbance score.

TABLE 4 Statistically significant ( $p < 0.05$ ) correlations between hippocampal subfield volumes and clinical measures in ME/CFS<sub>ICC</sub>

Hippocampal subfield	Clinical measure	r	p	df
Left hippocampal tail	fatigue	-0.803	0.016	10
Left hippocampal tail	SDS	0.762	0.004	10
Left CA4 body		0.590	0.043	10
Right hippocampal tail		0.706	0.010	10
Right subiculum body		0.618	0.032	10
Left GC-ML-DG head	phys_all	-0.688	0.013	10
Left CA4 head		-0.654	0.021	10
Left GC-ML-DG head	pain	-0.673	0.016	10
Left CA4 head		-0.650	0.022	10
Left subiculum head	procinfo	0.715	0.009	10
Left CA1 head		0.657	0.020	10
Left molecular layer HP head		0.694	0.012	10
Left whole hippocampal head		0.682	0.015	10
Right subiculum head		0.817	0.001	10

Note: The Spearman correlation test was used to perform the correlation analysis using SPSS software version 27.

Abbreviations: df, degree of freedom; phys\_all, SF36 physical score; procinfo, SF36 information processing score; r, correlation coefficient; SDS, sleep disturbance score.

(139.35 ± 18.34;  $r(19) = -0.487$ ,  $N = 24$ ,  $p = 0.025$ ). There were no statistically significant associations between pain, phys\_all, and procinfo with hippocampal subfield volumes in ME/CFS<sub>Fukuda</sub> patients.

### 3.3.2 | ME/CFS<sub>ICC</sub>

For ME/CFS<sub>ICC</sub> patients, hippocampal subfield volumes were significantly associated with clinical measures of “fatigue,” “SDS,”

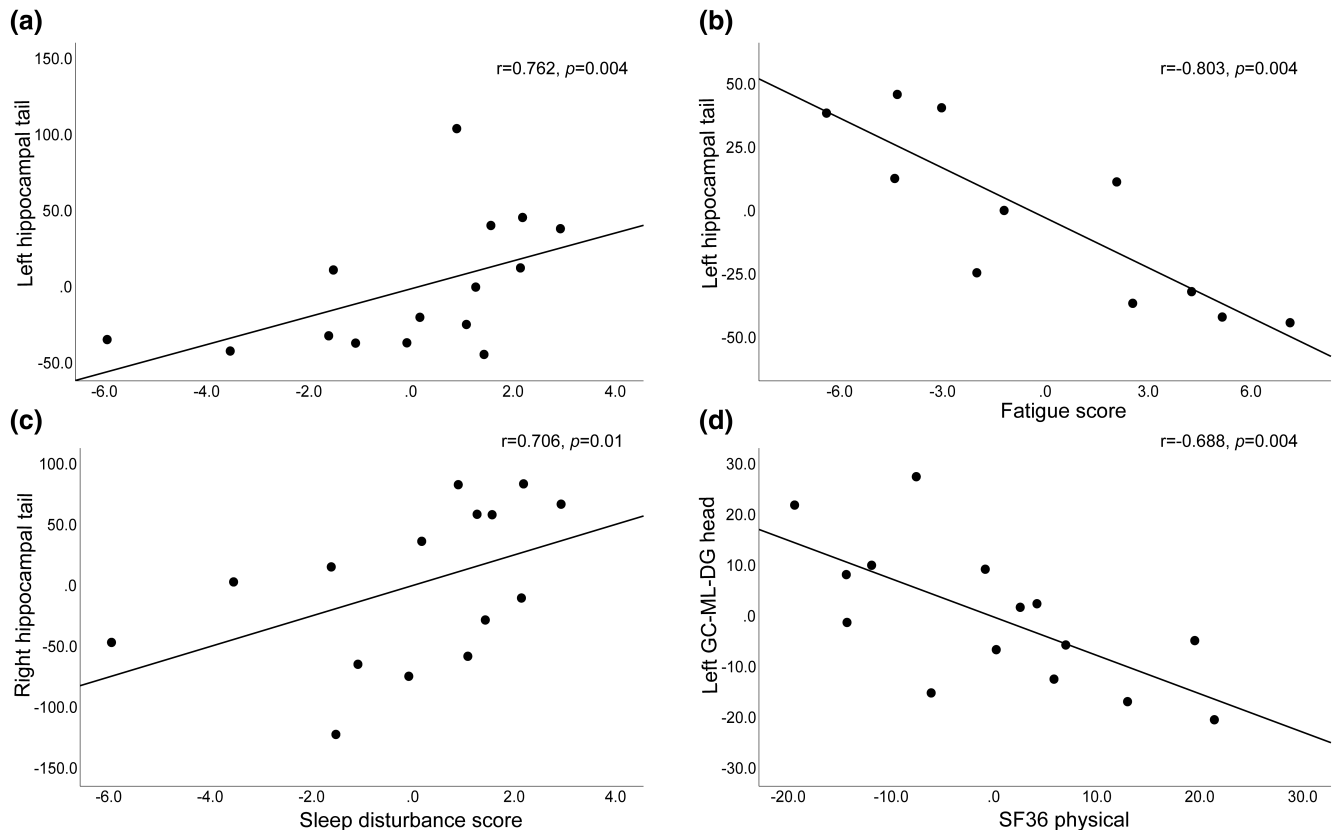
“phys\_all,” “procinfo,” and “pain” (see Table 4). We observed a statistically significant negative relationship between “fatigue” and left hippocampal tail volume (582.93 ± 46.76;  $r(6) = -0.803$ ,  $N = 16$ ,  $p = 0.016$ ) (see Figure 2). There was also a strong positive relationship between “SDS” and volumes of the left hippocampus tail (574.85 ± 35.00;  $r(10) = 0.762$ ,  $N = 16$ ,  $p = 0.004$ ), right hippocampus tail (587.52 ± 73.81;  $r(10) = 0.706$ ,  $N = 16$ ,  $p = 0.01$ ) (see Figure 2), and subiculum body volume (250.84 ± 28.32;  $r(10) = 0.618$ ,  $N = 16$ ,  $p = 0.032$ ). There was also a strong, negative relationship between “phys\_all” and volumes of the left GC-ML-DG head (160.12 ± 11.73;  $r(10) = -0.688$ ,  $N = 16$ ,  $p = 0.013$ ) (see Figure 2), and left CA4 head (135.13 ± 11.18;  $r(10) = -0.654$ ,  $N = 16$ ,  $p = 0.021$ ). We observed a strong, negative relationship between “pain,” left GC-ML-DG head (160.12 ± 11.73;  $r(10) = -0.67$ ,  $N = 16$ ,  $p = 0.016$ ), and left CA4 head volume (135.13 ± 11.18;  $r(10) = -0.65$ ,  $N = 16$ ,  $p = 0.022$ ) (see Figure 3). The “procinfo” clinical measure showed strong positive associations with volumes of the left subiculum head (210.91 ± 25.31;  $r(10) = 0.715$ ,  $N = 16$ ,  $p = 0.009$ ) (see Figure 3), CA1 head (560.53 ± 59.69;  $r(10) = 0.657$ ,  $N = 16$ ,  $p = 0.020$ ), molecular layer HP head (360.41 ± 35.34;  $r(10) = 0.694$ ,  $N = 16$ ,  $p = 0.012$ ), whole hippocampus head (1,851.10 ± 176.70;  $r(10) = 0.682$ ,  $N = 16$ ,  $p = 0.015$ ), and right subiculum head (207.04 ± 20.94;  $r(10) = 0.817$ ,  $N = 16$ ,  $p = 0.001$ ) (see Figure 3).

## 4 | DISCUSSION

We report differences in hippocampal subfield volumes and correlations with clinical measures in ME/CFS. Volume differences relative to HC were detected in more subfields in ME/CFS<sub>ICC</sub> than in ME/CFS<sub>Fukuda</sub> defined patients. Subiculum and presubiculum volumes were significantly larger in ME/CFS<sub>ICC</sub> patients compared with HC, in contrast to the smaller volumes observed in neurodegenerative diseases. Correlations of hippocampal subfield volumes with clinical measures for the Fukuda ME/CFS patients were significant for only fatigue and SDS (see Table 3). However, for the ICC patients we detected strong negative or positive correlations between all clinical measures tested (fatigue, procinfo, pain, phys\_all, and SDS) and multiple hippocampal subfield volumes (see Table 4). To our knowledge, this is the first study to investigate volumetric differences from HC in the hippocampal subfields of patients meeting Fukuda and ICC case definitions of ME/CFS. We demonstrated that a larger number of subfield volumes are different for the stricter ICC case definition ME/CFS<sub>ICC</sub> patients, and they exhibited significant relationships with more clinical measures.

### 4.1 | Group comparisons

Our study found significant volume differences between ME/CFS patients and HC in multiple hippocampus subfields. It has been reported that 89% of ME/CFS patients have memory and concentration



**FIGURE 2** Examples of strong correlations between hippocampal subfield volumes and clinical measures for ME/CFS<sub>ICC</sub>. We observed statistically significant relationship between hippocampal subfield volumes, “Sleep disturbance score” (a and c), fatigue (b), and SF36 physical score (d). Volumes for the shown subfields and clinical measures were residuals adjusted for age, sex, and ICV

problems, and difficulties in processing complex information (Jason et al., 1999). Previously, it has been shown that hippocampal subfield volumes are associated with memory ability (Daugherty et al., 2017). Our study showed *larger* subiculum, parasubiculum, and fimbria volumes in ME/CFS<sub>ICC</sub>. In contrast, neurodegenerative studies have reported *smaller* subiculum, parasubiculum, and presubiculum volumes in mild cognitive impairment and Alzheimer's disease (Carlesimo et al., 2015; Hanseeuw et al., 2011), bipolar disorder (Janiri et al., 2019), multiple sclerosis (Gold et al., 2010), and schizophrenia (Mathew et al., 2014) when compared with HC (Roddy et al., 2019). The larger subiculum and presubiculum in ME/CFS<sub>ICC</sub> patients suggest a neuroregulatory rather than a neurodegenerative response may be responsible in ME/CFS. Unexpected *increases* in myelination in the sensorimotor cortex which were inversely proportional to brainstem decreases were interpreted as evidence of a regulatory mechanism that maintains adequate brainstem–cortical communication (Barnden et al., 2018; Thapaliya et al., 2020, 2021). Edlow et al. (2016) reported rich connections between the subiculum and midbrain dorsal Raphe nucleus of the reticular activation system (RAS) in healthy participants. In a functional MRI study of the ME/CFS cohort examined here Barnden et al. (2019) reported strong RAS medulla connectivity with the right subiculum at rest, but impaired connectivity to the left subiculum during a cognitive task. We speculate that larger subiculum and presubiculum subfields in ME/

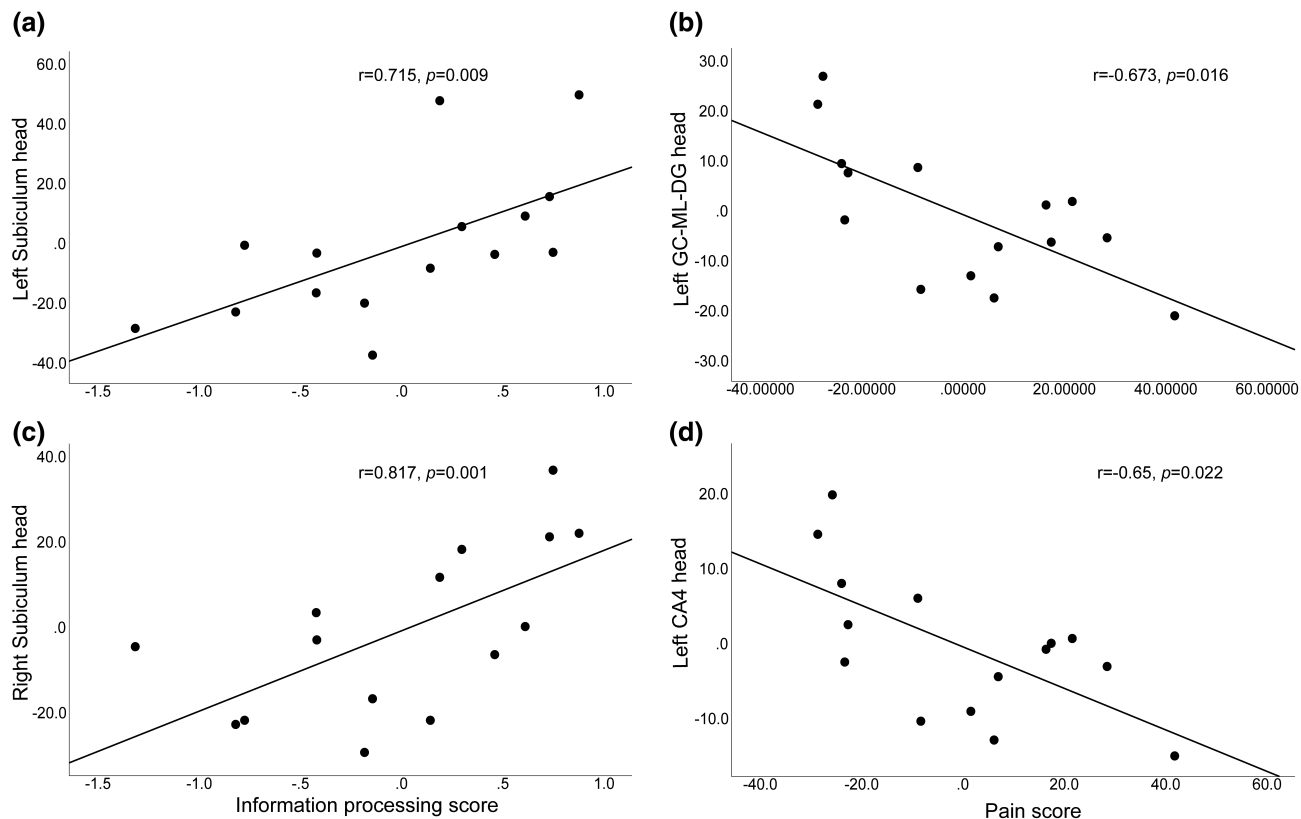
CFS<sub>ICC</sub> patients may also be due to the above-mentioned compensatory response to brainstem deficits (Barnden et al., 2018). The observation of impaired subiculum–brainstem connectivity (Barnden et al., 2019) may also promote upregulation in the hippocampus–brainstem system.

The fimbria is a white matter region from which axons project through a polysynaptic pathway to the cortex (Duvernoy, 2005). Importantly, a recent MRI study has suggested increased myelination in subcortical white matter regions in ME/CFS patients (Thapaliya et al., 2020). Moreover, an MRI diffusion study has shown that MD value was lower in the brainstem in ME/CFS<sub>ICC</sub> patients which is also consistent with increased subcortical myelination (Thapaliya et al., 2021). Collectively, these data suggest the fimbria volume was increased in ME/CFS<sub>ICC</sub> patients compared with HC. However, due to the paucity of literature, further comparative investigations of volumetric changes in hippocampal subfields need to be undertaken in ME/CFS patients.

## 4.2 | Correlations with clinical measures

Multiple significant subfield volume correlations were detected with clinical measures (Tables 3 and 4). Of the 22 significant correlations, 16 were detected on the left-hand side. Insofar as volume is a





**FIGURE 3** Some example plots of strong correlations between hippocampal subfield volumes and clinical measures for ME/CFS<sub>ICC</sub>. We observed statistically significant relationship between hippocampal subfield volumes, “information processing score” (a and c) and pain score (b and d). Volumes for the shown subfields and fatigue were residuals adjusted for age, sex, and ICV

surrogate for a functionally relevant feature such as myelination or axonal density, a subfield volume correlation with a clinical measure suggests this feature affects the control circuits that traverse the subfield and influence the clinical measure. This mechanism was proposed in an earlier MRI study of autonomic correlations (Barnden et al., 2016).

#### 4.2.1 | Fatigue

Fatigue is the primary symptom that affects the daily functioning of ME/CFS patients (Cockshell & Mathias, 2010). Our study shows a moderate ( $0.4 < r < 0.59$ ) positive correlation between “fatigue” and hippocampus subfield volumes (left: hippocampus tail, parasubiculum, HATA, whole hippocampus head, whole hippocampus, and right: CA1 head and molecular layer HP head) in ME/CFS<sub>Fukuda</sub> and a strong negative correlation with left hippocampus tail volume in ME/CFS<sub>ICC</sub> patients. A positive correlation means more severe fatigue is associated with larger volumes and vice versa. A functional MRI study of ME/CFS<sub>Fukuda</sub> patients showed a significant positive correlation between left hippocampus activation and fatigue (Boissoneault et al., 2016) although fatigue was significantly associated with total hippocampus volume reduction (Wasson et al., 2019).

#### 4.2.2 | Sleep deprivation

We observed a strong positive correlation between “SDS” and four hippocampus subfield volumes in ME/CFS<sub>ICC</sub> patients, with larger volumes associated with more severe SDS, while in ME/CFS<sub>Fukuda</sub> patients we observed a moderate negative correlation with a different subfield volume (right CA1 body) indicating smaller volume is associated with more severe SDS. A positive correlation means more severe SDS is associated with larger volumes and vice versa. Most ME/CFS patients exhibit sleep disturbances (Mariman et al., 2013) and impaired sleep homeostasis has been reported in patients with ME/CFS (Decker et al., 2009). In insomnia patients, worse sleep quality was associated with smaller CA1 and CA3 subfield volumes (Joo et al., 2014; Neylan et al., 2010) consistent with our ME/CFS<sub>Fukuda</sub>. Another study reported that sleep deprivation negatively impacts long-term potentiation in the hippocampal subfields (Alkadhi et al., 2013).

#### 4.2.3 | Physical activity

We found that “phys\_all” and two hippocampal subfield volumes are negatively correlated indicating that smaller subfield volumes are associated with greater physical activity (phys\_all). Patients with ME/

CFS have limited capacity for physical activity because of their post-exertional malaise (Yoshiuchi et al., 2007). Physical activity elevates brain-derived neurotrophic factor (BDNF) in the hippocampus that is necessary for neuroplasticity (Chen et al., 2008). Interestingly reduced BDNF in the hippocampus has been reported in ME/CFS patients (Chen et al., 2008).

#### 4.2.4 | Pain

Pain is another major symptom of ME/CFS (Bourke et al., 2014). Our study showed a strong negative relationship between "pain" and hippocampal subfield volumes in ME/CFS<sub>ICC</sub> patients indicating that smaller subfield volumes are associated with higher pain level. Previous studies in human and animal models have shown the involvement of the hippocampus in pain processing (Bingel et al., 2002; Schweinhardt et al., 2006; Zimmerman et al., 2009). Study in elderly population showed that a higher level of pain was associated with reduction of hippocampus subfield (CA4 and dentate gyrus) volumes but only in women (Ezzati et al., 2014) and was also associated with SF36 reported bodily pain (Zimmerman et al., 2009). A functional MRI study showed activation of the hippocampus region in response to pain stimuli (Bingel et al., 2002).

#### 4.2.5 | Information processing

ME/CFS patients have impaired memory and cognitive function (Carruthers et al., 2011) which is consistent with our study. We observed a strong positive correlation between "procinfo" and multiple hippocampus subfield volumes (Table 4) in ME/CFS<sub>ICC</sub> patients with larger volumes associated with poorer information processing score. Our recent findings with DTI parameters on the same cohort also showed abnormal regressions with "procinfo" in the hippocampus (Thapaliya et al., 2021). A study on mild cognitive impairment and Alzheimer's disease showed a positive relationship between total hippocampus volume and MMSE score (Peng et al., 2014). Another study in Alzheimer's disease showed a positive correlation between constructional recall score and CA1 and subiculum volumes (Lim et al., 2012) and memory test outcome was also positively associated with left hippocampus volume (Hardcastle et al., 2020).

#### 4.2.6 | Fukuda and ICC differences

Our study did not show statistically significant volume reduction in ME/CFS<sub>Fukuda</sub> patients when compared with HC after multiple comparison correction. However, we found volumetric differences in multiple hippocampal subfields in ME/CFS<sub>ICC</sub> patients. The correlation analysis of hippocampal subfield volumes in ME/CFS<sub>Fukuda</sub> patients showed a significant relationship for only two clinical measures whereas ME/CFS<sub>ICC</sub> patients showed significant correlations with

five clinical measures. These differences between ME/CFS<sub>Fukuda</sub> and ME/CFS<sub>ICC</sub> are likely due to the selection criteria. The Fukuda classification requires patients to meet fatigue severity criteria and exhibit another four of eight symptoms, but does not include the specific neurocognitive, cardiorespiratory, or thermoregulatory impairments now required in the ICC criteria (Carruthers et al., 2011). In addition, some symptoms of Fukuda overlap with depression (Carruthers et al., 2011). Therefore, the "Fukuda" group will contain patients without critical symptoms required by ICC and would not meet the currently accepted definition (Brown et al., 2013). Our recent study (Thapaliya et al., 2021) using the diffusion tensor imaging method did not show any significant differences between ME/CFS<sub>Fukuda</sub> patients and HC whereas significant differences were found between ME/CFS<sub>ICC</sub> patients compared to HC (Thapaliya et al., 2021).

#### 4.3 | Limitations

This study does have some limitations. Relatively small sample size in ME/CFS<sub>ICC</sub> might affect the power of the study to detect all the differences in hippocampal subfields and their association with clinical measures. Another limitation is that some of the clinical scores in this study were obtained by questionnaires, which by their subjective nature may limit interpretation of our findings. This study was a cross-sectional study. Longitudinal studies should be performed to test for progressive volumetric changes in hippocampal subfields in ME/CFS patients.

### 5 | CONCLUSION

Our study detected significant volumetric differences in hippocampal subfields in ME/CFS patients compared to HC. The group comparison revealed a larger number of subfield volumes were different in ME/CFS<sub>ICC</sub> than in ME/CFS<sub>Fukuda</sub> defined patients compared with HC. Therefore, volumetric changes in the hippocampus depend on patient selection criteria and demonstrate the importance of strict case definitions for ME/CFS patients. Unlike neurodegenerative diseases, some subfield volumes were greater in ME/CFS. The left subiculum, presubiculum head, and fimbria volumes were larger in ME/CFS patients who met ICC criteria. Clinical measures related to cognitive function (procinfo), pain, and physical activity showed a strong relationship with hippocampal subfield volumes only in the ME/CFS patients meeting only ICC criteria. Hippocampus subiculum volumes may be an imaging diagnostic biomarker for ME/CFS.

#### DECLARATION OF TRANSPARENCY

The authors, reviewers and editors affirm that in accordance to the policies set by the *Journal of Neuroscience Research*, this manuscript presents an accurate and transparent account of the study being reported and that all critical details describing the methods and results are present.



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## CONFLICT OF INTEREST

The authors report no conflict of interest.

## AUTHOR CONTRIBUTIONS

Conceptualization, K.T.; Methodology, K.T. and L.B.; Formal Analysis, K.T.; Writing - Original Draft, K.T.; Writing - Review & Editing, K.T., L.B., D.S., S.M.G., and J.S.; Supervision, L.B., D.S., and S.M.G.

## PEER REVIEW

The peer review history for this article is available at <https://publons.com/publon/10.1002/jnr.25048>.

## DATA AVAILABILITY STATEMENT

The study data are available upon reasonable request and following approval from the study group.

## ORCID

Kiran Thapaliya  <https://orcid.org/0000-0002-7667-770X>

Leighton Barnden  <https://orcid.org/0000-0002-3976-189X>

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## SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

**TABLE S1** Shows the mean and standard deviation of hippocampal subfield volumes for ME/CFS<sub>Fukuda</sub> patients and healthy controls (HC). ↓ indicates smaller volume than HC and ↑ indicates larger volume than HC. The effect size was determined by partial eta squared ( $\eta^2$ ). Bold indicates significant volume differences

**TABLE S2** Shows the mean and standard deviation of hippocampal subfield volumes for ME/CFS<sub>ICC</sub> patients and healthy controls (HC). ↓ indicates a smaller volume in ME/CFS<sub>ICC</sub> than HC and ↑ indicates a larger volume in ME/CFS<sub>ICC</sub> than HC. The effect size was determined by partial eta squared ( $\eta^2$ ). Bold indicates significant volume differences

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