Brain microstructure is linked to cognitive fatigue in early multiple sclerosis.

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Potential Conflicts of Interest

The authors have no conflict of interest to disclose.

Abstract

Cognitive fatigue is a major symptom of Multiple Sclerosis (MS), from the early stages of the disease. This study aims to detect if brain microstructure is altered early in the disease course and is associated with cognitive fatigue in people with MS (pwMS) compared to matched healthy controls (HC). Recently diagnosed pwMS (N=18, age <45 years old) with either a Relapsing-Remitting or a Clinically Isolated Syndrome course of the disease, and HC (N=19) matched for sex, age and education were analyzed. Quantitative multiparameter maps (MTsat, PD, R1 and R2*) of pwMS and HC were calculated. Parameters were extracted within the normal appearing white matter, cortical grey matter and deep grey matter (NAWM, NACGM and NADGM, respectively). Bayesian T-Test for independent samples assessed between-group differences in brain microstructure while associations between score at a cognitive fatigue scale and each parameter in each tissue class were investigated with Generalized Linear Mixed Models. Patients exhibited lower MTsat and R1 values within NAWM and NACGM, and higher R1 values in NADGM compared to HC. Cognitive fatigue was associated with PD measured in every tissue class and to MTsat in NAWM, regardless of group. Disease-specific negative correlations were found in pwMS in NAWM (R1, R2*) and NACGM (R1). These findings suggest that brain microstructure within normal appearing tissues is already altered in the very early stages of the disease. Moreover, additional microstructure alterations (e.g. diffuse and widespread demyelination or axonal degeneration) in pwMS may lead to disease-specific complaint of cognitive fatigue.

Keywords : Early Multiple Sclerosis; Cognitive Fatigue; Quantitative MRI; Fatigue ; Clinically Isolated Syndrome

Introduction

Fatigue is highly prevalent in multiple sclerosis (MS), already at the early stages of the disease[1,2]. Yet, the underlying mechanisms of fatigue are not elucidated. A central cause for MS-fatigue has often been proposed[3–5]. Accordingly, it was shown that fatigue in people with MS (pwMS) is associated with reduced fractional anisotropy in numerous white matter tracts and cortical and deep grey matter atrophy[6–9]. This is also supported by functional MRI studies suggesting that fatigue arises from networks reorganization due to structural alterations (see ARM et al., 2019 for a review[6]). However, additional evidence is needed to better understand fatigue pathophysiology.

Recently, advanced MRI techniques allowed for the quantification of brain tissue MR properties, which provide insight in tissue microstructure. These parameters are highly relevant in MS because they reveal subtle alterations in composition of lesions and normal appearing brain tissue microstructure that are not detected by conventional structural MRI[10]. In particular, longitudinal and transverse effective relaxation rates (respectively R1 = 1/T1 and R2* = 1/T2*) as well as Magnetization Transfer (MT) are sensitive measures of myelin and iron contents, which are altered in normal appearing tissue of pwMS [10–14]. Surprisingly, only a handful of studies assessed the potential link between brain microstructure and fatigue symptom in pwMS. Overall, T1 relaxation time increased (i.e. R1 decreased) in proportion to fatigue, especially in the deep grey matter, suggesting that fatigue results from demyelination and axonal loss[6,15]. In contrast, these studies did not find evidence for a link between MT and MS-fatigue[6,15,16]. Consequently, further investigation of brain microstructure in relation to fatigue symptom is needed. Especially, little is known about structural substrates of fatigue in the early stages of the disease, during which conventional MRI measures of brain alterations usually show mild lesion load and essentially no atrophy.

The objectives of this study are twofold. We will first explore the alterations of brain microstructure in the very early stages of the disease using multiparametric quantitative MRI, as previous studies suggest that microstructure abnormalities occur early in the disease[16–19]. Next, we will assess whether cognitive fatigue in pwMS is associated with altered brain microstructure, in comparison to healthy controls (HC). Identifying specific microstructural alterations relating to cognitive fatigue would improve our understanding of MS-fatigue mechanisms in the early stages of the disease.

Methods

Participants

Nineteen recently diagnosed pwMS and 19 HC matched for sex, age and education were recruited for this study at the neurology outpatient clinic of the University hospital in Liège, from May 2019 to August 2021 (Table 1). Eighteen patients were analyzed in this protocol. Patients presented either a Relapsing-Remitting (RR) or a Clinically Isolated Syndrome (CIS) course of the disease, according to the 2017 McDonald criteria[20]. Inclusion criteria for pwMS were absence of relapse in the last 6 months, disease duration below or equal to 5 years and a score at the Expanded Disability Status Scale (EDSS[21]) under 4.

Exclusion criteria for both groups included the existence of other neurological or psychiatric diseases, a history of mild or severe traumatic brain injury, and age above 45 years old. Lesion fraction (lesion volume to total intracranial volume [ICV]), as well as qMRI values (MTsat, PD, R1 and R2*) for lesion class are indicated in Table 1 and 3, respectively. In each pwMS and for each quantitative map, the score corresponds to the median value for all voxels considered as lesion tissue. This study was part of a wider research project (the FCSEP project), which full protocol is openly available on OSF (https://osf.io/egr6d/?view_only=cdcc343c7d71406685b038e46e88145b).

Table 1. Demographics and Cognitive Fatigue Scale

	pwMS N = 18	HC N = 19	BF_{10} T-Tests
Age, y, mean (SD)	31.28 (5.21)	31.42 (5.76)	*0.319*
Female, n (%)	13 (72.22)	14 (73.68)	0.350 [a]
Education, y, mean (SD)	14.39 (2.03)	14.63 (1.54)	0.340
Disease Duration, y, mean (SD)	1.79 (1.25)	n.a.	n.a.
EDSS, median (range)	1.75 (1 - 3)	n.a.	n.a.
FSMCcog, mean (SD)	29.50 (10.55)	25.74 (8.15)	1.394
BDI score, mean(SD)	12.11 (11.49)	10.74 (10.29)	0.337
Lesion fraction (%), mean (SD) [range]	0.44(0.46) [0.03-1.45]	/	/

Disease Duration: years since diagnosis; FMSCcog: cognitive sub-scale of the Fatigue Scale for Motor and Cognitive Functions; BDI: Deck Depression Inventory; pwMS: people with Multiple Sclerosis; HC: Healthy Controls. Results in italic exhibits moderate evidence for an absence of effect ($BF_{10} < .333$). [a]Contingency table analysis for independent multinomial sampling[23]

Standard Protocol Approvals, Registrations, and Patient Consents

The whole study was approved by the Ethic Committee of the University Hospital in Liège (approval number B707201835630). Each participant signed informed consent form before starting the study.

Materials

Participants completed the Fatigue Scale for Motor and Cognitive Functions (FSMC[22]), a scale which comprises sub-scores for the cognitive and physical modalities of fatigue symptom in everyday life (trait fatigue). Details of the MPM protocol acquisition performed for each participant on a 3 tesla MRI are available at https://osf.io/egr6d/?view_only=cdcc343c7d71406685b038e46e88145b. Briefly, 3 co-localized 3 dimensional 1 mm isotropic resolution multi-echo fast low angle shot data sets were acquired with predominantly proton density weighted (PDw), T1 weighting (T1w) and MT weighting (MTw). Calibration sequences to correct for B0 and B1 transmit/receive field inhomogeneities were also acquired. One participant from the pwMS group had to be excluded due to poor quality of MRI data (head motion artifacts).

MR image processing

All data processing was performed in Matlab (The MathWorks Inc., Natick, MA, USA) using SPM12 (www.fil.ion.ucl.ac.uk/spm) and two additional dedicated SPM extensions: the "quantitative MRI and in

vivo histology using MRI" toolbox (hMRI, http://hmri.info)[24], and "US-with-Lesion" tool (USwL, https://github.com/CyclotronResearchCentre/USwLesion). Summary parameters, mean and variance within white matter, were provided by the hMRI toolbox for each subject's quantitative maps to ensure that signal value is within acceptable range.

Quantitative Magnetic Resonance Imaging (qMRI) maps (MTsat, PD, RI and R2*) were estimated using the hMRI toolbox. T1w, PDw and MTw gradient echo images acquired at multiple echo times (TE) were extrapolated to TE=0 to increase signal-to-noise ratio in the modelled maps and remove the otherwise remaining R2* bias[10,24,25]. The TE=0 extrapolated MTw, PDw and T1w images were used to calculate MT saturation, RI and apparent signal amplitude A* maps. PD maps were derived from A* maps, which are proportional to proton density. All quantitative maps were corrected for inhomogeneities from local RF transmit field (B1+), and RI quantitative maps were further corrected for imperfect RF spoiling using the strategy of Preibisch and Deichmann[26]. The received bias field map (B1-) was used to correct PD maps for instrumental biases. The R2* map was estimated from all three multi-echo series (MTw, PDw and R1w) using the ESTATICS model[25].

Segmentation and normalization to MNI space was performed with the standard "Unified Segmentation" (US)[27] algorithm from SPM for HC, and its extension USwL for patients[28]. Multi-channel segmentation was conducted, using T1w extrapolated at TE=0 and FLAIR images.

The method generated the segmented tissue classes (*a posteriori* tissue, including lesion probability maps), as well as spatial warping into standard template space. Segmentation teased out the different tissue classes of interest: NAWM, NACGM, NADGM and lesions (in the case of pwMS).

Statistical Analyses

To analyze the microstructure within tissue classes, *a posteriori* tissue maps were binarized and tissue-specific independent masks were constructed: each voxel was assigned to one single tissue class with the highest probability for that voxel (provided that this probability was above 0.2). Images and their segmented tissues were kept in subject's space, quantitative MR parameters were extracted from all voxels of a given tissue class, and their median value was calculated.

Between-group differences regarding qMRI median values in each tissue class were assessed by means of Bayesian T-tests using JASP (Jeffreys's Amazing Statistics Program v.0.16; https://jasp-stats.org). Results are reported in terms of Bayes Factor (BF) which corresponds to the likelihood ratio of evidence provided by the data over two hypotheses. Significant results were determined using Jeffreys's grades of evidence, as described in Table 2[29].

Table 2. Jeffreys's Bayes factor evidence category

BF_{10}	Interpretation
>100****	Extreme evidence for H1
30–100***	Very Strong evidence for H1
10–30**	Strong evidence for H1
3–10*	Moderate evidence for H1
1–3	Anecdotal evidence for H1
1	No evidence
0.333–1	Anecdotal evidence for H0
0.1–0.333*	Moderate evidence for H0
0.033–0.1**	Strong evidence for H0
0.01–0.033***	Very Strong evidence for H0
<0.01****	Extreme evidence for H0

Level of evidence for the alternative (H1) and the null (H0) hypothesis depending on Bayes Factor (BF_{10} here). For instance, a BF_{10} of 5 indicates that the data observed are 5 times more likely to happen under the alternative hypothesis rather than the null, corresponding to a moderate evidence for H1.

Frequentists Generalized Linear Mixed Model (GLMM) analyses were performed in SAS 9.4 (SAS Institute, Cary, NC). For each qMRI parameter and tissue class individually, a separate GLMM tested the effect of age, gender, as well as group (HC vs pwMS), median MPM value and their interaction (i.e., group*median MPM value), on the cognitive FSMC score. Anticipating on the result section, the BDI score was not included as a confound in the analyses due to its high collinearity with the cognitive fatigue score. Statistical significance was estimated at $p< .05$. In total, twelve models were tested (3 tissue classes x 4 parameters) and a False Discovery Rate (FDR) correction was implemented to account for multiple comparisons[30]. Effect sizes of significant results were estimated with semi-partial R^2 [31].

Data availability

Anonymized data not published within this article are not publicly available due to privacy or ethical restrictions but will be made available upon reasonable request from any qualified investigator.

Results

Comparison of scores at the BDI and cognitive subscale of the FSMC showed inconclusive results for between-group difference (T-tests; $3 < BF_{10} <.333$; see Table 2). Seven (37%) patients reached the FSMC cutoff score for severe cognitive fatigue, 3 (16%) for moderate fatigue and the remaining 10 (52%) had normal to mild fatigue (range scores: 41-43; 28-33; 10-27, respectively). In HC, 2 (11%) participants had severe cognitive fatigue, 6 (32%) had moderate fatigue, and the remaining 11 (58%) had normal to mild fatigue symptoms (range scores: 36-44; 29-32; 11-27, respectively). Pearson correlations analyses between depression and fatigue score showed strong evidence for an association in pwMS ($r=0.62$, $10 < BF_{10} <30$) and very strong evidence in HC ($r=0.73$, $30 < BF_{10} <100$).

T-tests assessing between-group differences in qMRI parameters (see Tabel 3) displayed moderate to strong evidence for a between-group difference for MTsat in the NAWM ($BF_{10} >3$) and NACGM ($BF_{10} >10$),

with patients exhibiting lower values of MTsat (Table 3). Between-group difference in the RI parameter was found in all tissue classes, and was supported by moderate to strong evidence (all BFs_{10} >3), with pwMS showing lower values than HC in NAWM and NACGM but higher values in NADGM. Moderate evidence for an absence of between-group difference was found for PD in the NAWM and R2* in NADGM (both BFs_{10} <.333). Other results were inconclusive (3 < BF_{10} <.333).

Table 3. Between-group differences in qMRI parameters

	pwMS N = 18	HC N = 19	BF_{10}
MTsat	mean (SD)	mean (SD)	
NAWM	1.67 (0.06)	1.73 (0.08)	**3.14***
NACGM	0.85 (0.02)	0.88 (0.04)	**10.17****
NADGM	0.96 (0.05)	0.94 (0.04)	0.62
Lesion	1.23 (0.24)	N/A	
PD			
NAWM	69.02 (0.18)	69.03 (0.19)	*0.328*
NACGM	73.43 (0.95)	80.03 (1.09)	1.10
NADGM	79.04 (1.30)	80.02 (1.20)	2.66
Lesion	73.67 (2.01)	N/A	
RI			
NAWM	1.01 (0.03)	1.06 (0.06)	**11.20****
NACGM	0.65 (0.01)	0.70 (0.06)	**23.73****
NADGM	0.74 (0.02)	0.69 (0.07)	**8.23***
Lesion	0.87 (0.09)	N/A	
*R2**			
NAWM	20.85 (0.52)	21.24 (0.74)	1.16
NACGM	16.53 (0.44)	16.65 (0.48)	0.40
NADGM	19.91 (1.25)	19.79 (1.23)	*0.330*
Lesion	18.32 (1.78)	N/A	

Results from between-group T-Tests analyses on qMRI parameters in (NAWM), Normal Appearing Cortical Grey Matter (NACGM) and Normal Appearing Deep Grey Matter (NADGM). * BF_{10} > 3: Moderate Evidence for H1; ** BF_{10} > 10 Strong Evidence for H1. Results in italic exhibits a moderate evidence for an absence of effect (BF_{10} < .333).

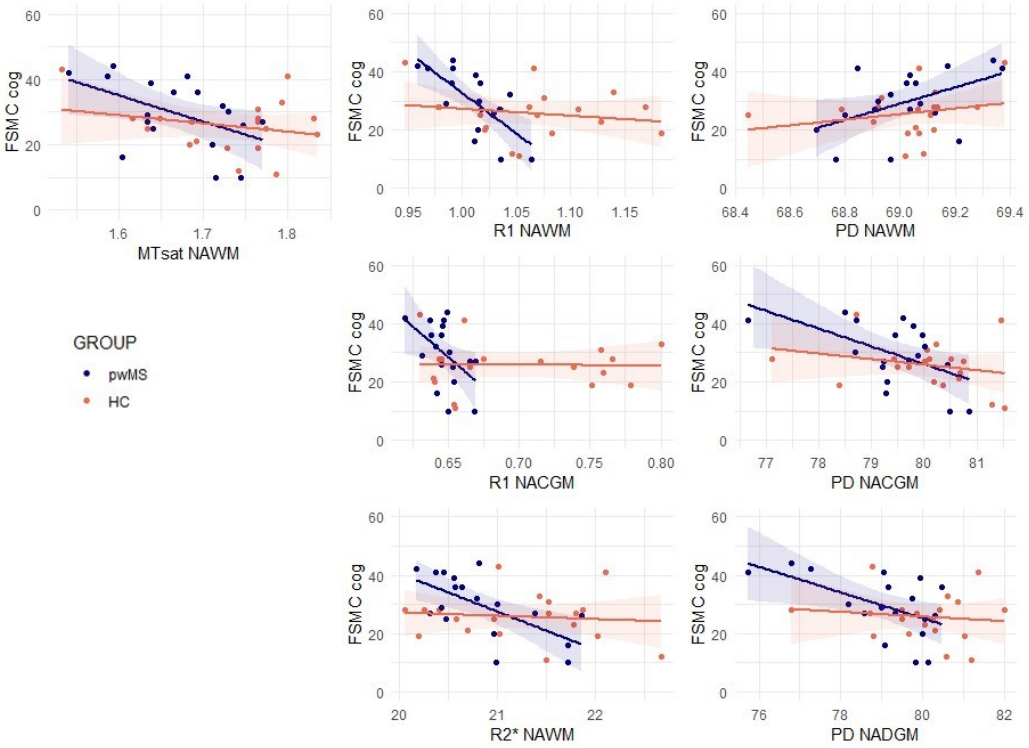
GLMMs testing the link between fatigue scores (cognitive subscale of the FSMC) and global MPM measurements showed significant results in the two groups (Table 4, with FDR correction): MTsat in NAWM (p < .05); PD in NAWM, NACGM and NADGM (all p < .05); RI in NAWM (p < .001) and NACGM (p < .01); R2* in NAWM (p < .05). A graphical representation of these associations is presented in Figure 1. The Parameter*Group interaction was significant for RI in the NAWM and the NACGM (both p < .01) and for R2* in the NAWM (p < .05) where parametric values were negatively associated with cognitive fatigue in patients only (middle panel in Figure 1).

Table 4. Results from GLMM analyses of the effects of group and MPM values on cognitive fatigue

MTsat	NAWM	NACGM	NADGM
Group	$F_{1, 31} = 1.36$ $p = .25$	$F_{1, 31} = 0.47$ $p = .50$	$F_{1, 31} = 1.66$ $p = .21$
MTsat	**$F_{1, 31} = 5.56$** **$p = .025$*** **$R^2_{\beta^*} = .15$**	$F_{1, 31} = 0.51$ $p = .48$	$F_{1, 31} = 0.83$ $p = .37$
MTsat*Group	$F_{1, 31} = 1.32$ $p = .26$	$F_{1, 31} = 0.43$ $p = 0.52$	$F_{1, 31} = 1.47$ $p = .23$
PD			
Group	$F_{1, 31} = 1.19$ $p = .28$	$F_{1, 31} = 1.20$ $p = .28$	$F_{1, 31} = 1.86$ $p = .18$
PD	**$F_{1, 31} = 5.39$** **$p = .027$*** **$R^2_{\beta^*} = .15$**	**$F_{1, 31} = 6.20$** **$p = .018$*** **$R^2_{\beta^*} = .17$**	**$F_{1, 31} = 5.39$** **$p = .027$*** **$R^2_{\beta^*} = .15$**
PD*Group	$F_{1, 31} = 1.20$ $p = .28$	$F_{1, 31} = 1.18$ $p = .28$	$F_{1, 31} = 1.84$ $p = .18$
R1			
Group	**$F_{1, 31} = 9.19$** **$p = .005$**** **$R^2_{\beta^*} = .23$**	**$F_{1, 31} = 9.11$** **$p = .005$**** **$R^2_{\beta^*} = .23$**	$F_{1, 31} = 1.56$ $p = .22$
R1	**$F_{1, 31} = 14.18$** **$p < .001$***** **$R^2_{\beta^*} = .31$**	**$F_{1, 31} = 9.43$** **$p = .004$**** **$R^2_{\beta^*} = .23$**	$F_{1, 31} = 1.51$ $p = .23$
R1*Group	**$F_{1, 31} = 9.15$** **$p = .005$**** **$R^2_{\beta^*} = .23$**	**$F_{1, 31} = 8.99$** **$p = .005$**** **$R^2_{\beta^*} = .22$**	$F_{1, 31} = 1.42$ $p = .24$
*R2**			
Group	**$F_{1, 31} = 4.71$** **$p = .038$*** **$R^2_{\beta^*} = .13$**	$F_{1, 31} = 2.45$ $p = .13$	$F_{1, 31} = 0.10$ $p = .75$
R2*	**$F_{1, 31} = 7.24$** **$p = .011$*** **$R^2_{\beta^*} = .19$**	$F_{1, 31} = 2.21$ $p = .15$	$F_{1, 31} = 2.62$ $p = .12$
R2**Group	**$F_{1, 31} = 4.63$** **$p = .039$*** **$R^2_{\beta^*} = .13$**	$F_{1, 31} = 2.36$ $p = .13$	$F_{1, 31} = 0.07$ $p = .80$

Results from GLMM analyses with cognitive fatigue as dependent variable and group, qMRI parameters (MTsat, PD, R1 or R2*) and their interaction as independent variables in the Normal Appearing White Matter (NAWM), Normal Appearing Cortical Grey Matter (NACGM) and Normal Appearing Deep Grey Matter (NADGM). *$p < .05$; ** $p < .01$; ***$p < .001$

Figure 1: Correlations between the cognitive sub-score of the FSMC and MTsat in NAWM (top row, left column), R1 in NAWM (top row, center column) and NACGM (middle row, center column), and R2* in NAWM (bottom row, center column) and PD in the three tissue classes (right column), pwMS patients are represented in blue, HC in orange.



Discussion

This study aimed at determining if trait cognitive fatigue is associated with microstructure integrity within normal appearing brain tissues and whether these associations differ between early pwMS and HC.

Brain Microstructure is Altered Early in Multiple Sclerosis

This study provides additional support suggesting that microstructure integrity is already altered during the first years of the disease, and occurs in both NAWM and normal appearing cortical grey matter (NACGM). In early pwMS, MTsat and R1 values showed significant small decreases in the NAWM and NACGM, as compared to controls, suggesting diffuse and widespread demyelination and/or axonal injury in both white and cortical grey matters[32-35]. These results are consistent with other studies showing decreased R1 and/or MT values in NAWM and NACGM in pwMS[10,13], even in the early stages of the disease[17,18].

Remarkably, within the deep gray matter, patients exhibited increased RI values whereas R2* was unchanged compared to controls. Conflicting microstructure alterations have been observed within the deep grey matter. R2* within NADGM was found to be increased in pwMS in some studies[36,37], but not in others[38,39]. Likewise, in contrast to the present study, recent articles did not report any change in RI in these structures[14,40]. In a previous study from our lab, MTsat in NADGM was found to be decreased in pwMS[10], while no between-group difference was observed regarding RI in this tissue class. Noteworthy, RI median values obtained by HC were higher in the study from Lommers et al.[10], and appeared to be closer to values obtained here for patients rather than controls (mean RI NADGM value in HC: 0.77 in Lommers et al.[10], in the present study: 0.69 for HC and 0.74 for patients). One critical difference between the two studies pertains to age. Indeed, participants are younger in the present study, focusing on the early stages of the disease. As increased RI values vary with iron concentration, and because brain iron deposition increases with age[41], we suggest that results obtained in our sample of pwMS reflect an early increase in iron concentration due to the disease[11]. Later on, the interplay between high iron concentration and demyelination within the NADGM could lead to similar RI values between pwMS and healthy subjects[10]. However, the heterogeneity of lesions in pwMS may also contribute to the conflicting results across studies and this disease progression-related explanation need confirmation in further studies.

However, a significant increase of iron level in the early stages of MS does not fit with normal NADGM R2*, as this parameter is also sensitive to iron[11]. Several studies pointed out that R2* values depend on disease duration, suggesting that iron deposits increase with years[36,37]. Similarly to RI, R2* is sensitive but not specific to iron and can reflect other mechanisms at play such as calcium concentration, fiber orientation and water content[12]. Additionally, Hernandez-Torres and colleagues[42] demonstrated that regional R2* augmentations *per se* are not necessarily linked to increased iron deposition in the context of MS, as atrophy can also influence R2* values. Consequently, DGM atrophy could play a role in the estimated link between R2* and disease duration that is usually interpreted as iron deposition.

Cognitive Fatigue Is Associated with Brain Microstructure

Median qMRI parameter values were associated with cognitive fatigue, after controlling for demographic variables. Results indicate that fatigue is associated with MTsat in NAWM and PD in all brain tissue studied, regardless of group. This suggests that cognitive fatigue is linked to brain microstructure, including in healthy subjects. Tentatively, we propose that this association relates to individual variability in brain integrity, myelination and neuronal density, in relation to brain reserve and neuronal plasticity[43-44]. For instance, the negative association found between fatigue and MTsat in NAWM in both groups could reflect global myelin density and integrity of the structural connectome. This would suggest that brain reserve, and by extent, cognitive reserve, would protect against cognitive fatigue, even in the absence of pathology. This hypothesis is supported by studies showing that years of education (a proxy for cognitive reserve) correlates negatively with fatigue scales[1,46] and would deserve further investigations.

The presence of associations between brain microstructure characteristics and cognitive fatigue regardless of disease state suggests that factors common to all our participants may also intervene. In a

comprehensive review, Penner and Paul[47] discussed factors contributing to fatigue in neurological disease. Some of the factors they identified may also be experienced transiently in healthy participants, such a poor sleep, inflammation or depression. Moreover, other factors such as nutrition (e.,g., lack of vitamins and minerals[48,49]) seems also influence fatigue level and may have effect on brain microstructure[50,51]. Finally, previous studies showed a frequent coexistence of fatigue and depression in pwMS[52,53]. In a recent study, cognitive fatigue is predicted by higher levels of depression and reduced microstructural tissue integrity in the cortico striatal thalamo cortical loop[52]. However, damage to the ventromedial and temporo-insular networks may also play a role in the development of fatigue independent of depression[54]. Depression status was not included in our statistical models due to high collinearity with cognitive fatigue score. Future studies should be interested to specify how presence of depressive symptoms in HC and pwMS may influence the association between brain microstructure characteristics and cognitive fatigue. For example, a recent study showed that depression predict the relationship between fatigue and information processing speed in pwMS using mediation analyses[55].

Cognitive fatigue was also related to brain microstructure in a disease-specific manner. Fatigue was negatively correlated to R1 in the NAWM and NACGM as well as R2* in the NAWM, only in pwMS. These results suggest that fatigue relates to demyelination, rather than local iron accumulation[56-59]. Similarly, demyelination of cortical NAGM likely explains the negative association between cognitive fatigue and NACGM R1[14]. Oddly enough, in the present study, no significant fatigue by group interaction was found for NADGM R1, in contrast to several other reports[6, 60]. One possible explanation for the absence of significant result, beside the small sample size, is that DGM alterations gradually accrue with disease duration and are preferably linked to fatigue in the later stages of MS.

Conclusion

Brain microstructure within normal appearing tissues is already altered in the very early stages of the disease, and suggests decreased myelin content in normal appearing white and cortical grey matters. Regarding the association between cognitive fatigue and brain microstructure, both general and disease-specific correlations were found, suggesting that fatigue in pwMS is determined by specific additional factors. More specifically, we tentatively propose that fatigue is determined by brain reserve and plasticity in the general population (including pwMS), and that in the context of MS, pathophysiological mechanisms causing microstructure alterations (e.g., diffuse and widespread demyelination or axonal degeneration) will lead to disease-specific cognitive fatigue. Hence, trait cognitive fatigue in pwMS seems specifically associated with global integrity of the NAWM and the NACGM. However, we have not observed association with integrity within the NADGM, which could suggest that structural alterations of the deep grey matter are involved in fatigue pathophysiology in the latter stages of the disease.

References

1. Weiland, T. J., Jelinek, G. J, Marck, C. H. *et al.* Clinically Significant Fatigue: Prevalence and Associated Factors in an International Sample of Adults with Multiple Sclerosis Recruited via the Internet. *PLoS One* **10**, (2015), DOI: 10.1371/journal.pone.0115541.

2. Bakshi, R., Shaikh, Z. A., Miletich, R. S. *et al.* Fatigue in multiple sclerosis and its relationship to depression and neurologic disability. *Mult. Scler.* **6**, 181–185 (2000), DOI: 10.1177/135245850000600308 .

3. Dobryakova, E., Genova, H. M., DeLuca, J. & Wylie, G. R. The dopamine imbalance hypothesis of fatigue in multiple sclerosis and other neurological disorders. *Frontiers in Neurology* **6**, 1–8 (2015), DOI: 10.3389/fneur.2015.00052.

4. Chaudhuri, A. & Behan, P. O. Fatigue in neurological disorders. *Lancet* **363**, 978–988 (2004), DOI: 10.1016/S0140-6736(04)15794-2.

5. Chaudhuri, A. & Behan, P. O. Fatigue and basal ganglia. *J. Neurol. Sci.* **179**, 34–42 (2000), DOI: 10.1016/s0022-510x(00)00411-1 .

6. Arm, J., Ribbons, K., Lechner-Scott, J. & Ramadan, S. Evaluation of MS related central fatigue using MR neuroimaging methods: Scoping review. *J. Neurol. Sci.* **400**, 52–71 (2019), DOI: 10.1016/j.jns.2019.03.007 .

7. Palotai, M. & Guttmann, C. R. G. Brain anatomical correlates of fatigue in multiple sclerosis. *Multiple Sclerosis Journal* **26**, 751–764 (2020), DOI: 10.1177/1352458519876032 .

8. Sander, C., Eling, P., Hanken, K., Klein, J., Kastrup, A. & Hildebrandt, H. The impact of MS-related cognitive Fatigue on Future Brain Parenchymal loss and relapse : a 17-Month Follow-up study. *Front. Neurol.* **7**, (2016), DOI: 10.3389/fneur.2016.00155.

9. Damasceno, A., Damasceno, B. P. & Cendes, F. Atrophy of reward-related striatal structures in fatigued MS patients is independent of physical disability. *Mult. Scler.* **22**, 822–829 (2016), DOI: 10.1177/1352458515599451.

10. Lommers, E., Simon, J., Reuter, G. *et al.* Multiparameter MRI quantification of microstructural tissue alterations in multiple sclerosis. *NeuroImage Clin.* **23**, 101879 (2019), DOI: 10.1016/j.nicl.2019.101879.

11. Neema, M., Stankiewicz, J., Arora, A. *et al.* T1- and T2-based MRI measures of diffuse gray matter and white matter damage in patients with multiple sclerosis. *J. Neuroimaging* **17**, 16–21 (2007), DOI: 10.1111/j.1552-6569.2007.00131.x.

12. Cohen-Adad, J. What can we learn from T2* maps of the cortex? *Neuroimage* **93**, 189–200 (2014), DOI: 10.1016/j.neuroimage.2013.01.023.

13. Filippi, M. & Agosta, F. Magnetization transfer MRI in multiple sclerosis. *J. Neuroimaging* **17**, 22–26 (2007), DOI: 10.1111/j.1552-6569.2007.00132.x.

14. Lommers, E., Guillemin, C., Reuter, G. *et al.* Voxel-Based quantitative MRI reveals spatial patterns of grey matter alteration in multiple sclerosis. *Hum. Brain Mapp.* **42**, 1003–1012 (2021), DOI: 10.1002/hbm.25274.

15. Zellini, F., Niepel, G., Tench, C. R. & Constantinescu, C. S. Hypothalamic involvement assessed by T1 relaxation time in patients with relapsing - Remitting multiple sclerosis. *Mult. Scler.* **15**, 1442–1449 (2009), DOI: 0.1177/1352458509350306.

16. Bonnier, G., Roche, A., Romascano, D. *et al.* Advanced MRI unravels the nature of tissue alterations in early multiple sclerosis. *Ann. Clin. Transl. Neurol.* **1**, 423–432 (2014), DOI: 10.1002/acn3.68.

17. Davies, G. R., Ramio-Torrenta, L., Hadjiprocopis, A. *et al.* Evidence for grey matter MTR abnormality in minimally disabled patients with early relapsing-remitting multiple sclerosis. *J. Neurol. Neurosurg. Psychiatry* **75**, 998–1002 (2004), DOI: 10.1136/jnnp.2003.021915.

18. Gracien, R. M., Reitz, S. C., Hof, S.-M. *et al.* Assessment of cortical damage in early multiple sclerosis with quantitative T2 relaxometry. *NMR Biomed.* **29**, 444–450 (2016), DOI: 10.1002/nbm.3486 .

19. Griffin, C. M., Chard, D. T., Parker, G. J.-M., Barker, G. J., Thompson, A. J. & Miller, D. H. The relationship between lesion and normal appearing brain tissue abnormalities in early relapsing remitting multiple sclerosis. *J. Neurol.* **249**, 193–199 (2002), DOI: 10.1007/pl00007864.

20. Thompson, A. J., Banwell, B. L., Barkhof, F. *et al.* Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. *Lancet Neurol.* **17**, 162–173 (2018), DOI: 10.1016/S1474-4422(17)30470-2.

21. Kurtzke, J. F. Rating neurologic impairment in multiple sclerosis: An expanded disability status scale (EDSS). *Neurology* **33**, 1444–1452 (1983), DOI: 10.1212/wnl.33.11.1444 .

22. Penner, I. K., Raselli, C., Stöcklin, M., Opwis, K., Kappos, L. & Calabrese, P. The Fatigue Scale for Motor and Cognitive Functions (FSMC): Validation of a new instrument to assess multiple sclerosis-related fatigue. *Mult. Scler.* **15**, 1509–1517 (2009), DOI: 10.1177/1352458509348519.

23. Jamil, T., Ly, A., Morey, R. D., Love, J., Marsman, M. & Wagenmakers, E.-J. Default "Gunel and Dickey" Bayes factors for contingency tables. *Behav. Res. Methods* **49**, 638–652 (2017), DOI: 10.3758/s13428-016-0739-8.

24. Tabelow, K., Balteau, E., Ashburner, J. *et al.* hMRI – A toolbox for quantitative MRI in neuroscience and clinical research. *Neuroimage* **194**, 191–210 (2019), DOI: 10.1016/j.neuroimage.2019.01.029.

25. Weiskopf, N., Callaghan, M. F., Josephs, O., Lutti, A. & Mohammadi, S. Estimating the apparent transverse relaxation time (R2*) from images with different contrasts (ESTATICS) reduces motion

artifacts. *Front. Neurosci.* **8**, 1–10 (2014), DOI: 10.3389/fnins.2014.00278.

26. Preibisch, C. & Deichmann, R. Influence of RF spoiling on the stability and accuracy of T1 mapping based on spoiled FLASH with varying flip angles. *Magn. Reson. Med.* **61**, 125–135 (2009), DOI: 10.1002/mrm.21776.

27. Ashburner, J. & Friston, K. J. Unified segmentation. *Neuroimage* **26**, 839–851 (2005), DOI: 10.1016/j.neuroimage.2005.02.018.

28. Phillips, C. U. de L.-Ul. > G.-C. I. vivo I. & Pernet, C. U. *Unifying lesion masking and tissue probability maps for improved segmentation and normalization*. *23rd Annual Meeting of the Organization for Human Brain Mapping* (2018).

29. Jeffreys, H. *Theory of Probability. 3rd Edition*. (Clarendon Press, 1961).

30. Benjamini, Y. & Hochberg, Y. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *J. R. Stat. Soc. Ser. B* **57**, 289–300 (1995).

31. Jaeger, B. C., Edwards, L. J., Das, K. & Sen, P. K. An R^2 statistic for fixed effects in the generalized linear mixed model. *J. Appl. Stat.* **44**, 1086–1105 (2017), DOI: 10.1080/02664763.2016.1193725.

NEW

32. Stüber C., Morawski M., Schäfer A., *et al.* Myelin and iron concentration in the human brain: a quantitative study of MRI contrast. *Neuroimage* **93**, 95–106, 2014, DOI: doi: 10.1016/j.neuroimage.

33. van der Weijden, C.W.J., García, D.V., Borra, R.J.H., *et al.* Myelin quantification with MRI: A systematic review of accuracy and reproducibility. *Neuroimage* **226**, 117561, 2021, DOI: 10.1016/j.neuroimage.2020.117561.

34. Schmierer, K., Tozer, D.J., Scaravilli, F., *et al.* Quantitative magnetization transfer imaging in postmortem multiple sclerosis brain. *J Magn Reson Imaging* **26**, 41-51, 2007, DOI: 10.1002/jmri.20984.

35. Laule, C., Pavlova, V., Leung, E., *et al.* Diffusely abnormal white matter in multiple sclerosis: further histologic studies provide evidence for a primary lipid abnormality with neurodegeneration. *J Neuropathol Exp Neurol* **72**, 42-52, 2013, DOI: 10.1097/NEN.0b013e31827bced3.

36. Khalil, M., Langkammer, C., Ropele, S. *et al.* Determinants of brain iron in multiple sclerosis: A quantitative 3T MRI study. *Neurology* **77**, 1691–1697 (2011), DOI: 10.1212/WNL.0b013e318236ef0e.

37. Ropele, S., Kilsdonk, I. D., Wattjes, M. P. *et al.* Determinants of iron accumulation in deep grey matter of multiple sclerosis patients. *Mult. Scler. J.* **20**, 1692–1698 (2014), DOI:

10.1177/1352458514531085.

38. Lommers, E. *MULTIPARAMETER MRI QUANTIFICATION OF MICROSTRUCTURAL BRAIN ALTERATIONS IN MULTIPLE SCLEROSIS*. (ULiège - Université de Liège [Applied Sciences], 2019).

39. Elkady, A. M., Cobzas, D., Sun, H., Seres, P., Blevins, G. & Wilman, A. H. Five year iron changes in relapsing-remitting multiple sclerosis deep gray matter compared to healthy controls. *Mult. Scler. Relat. Disord.* **33**, 107–115 (2019), DOI: 10.1016/j.msard.2019.05.028.

40. Andica, C., Hagiwara, A., Kamagata, K. *et al.* Gray Matter Alterations in Early and Late Relapsing-Remitting Multiple Sclerosis Evaluated with Synthetic Quantitative Magnetic Resonance Imaging. *Sci. Rep.* **9**, 1–10 (2019), DOI: 10.1038/s41598-019-44615-3.

41. Brass, S. D., Chen, N., Mulkern, R. V. & Bakshi, R. Magnetic Resonance Imaging of Iron Deposition in Neurological Disorders. *Top. Magn. Reson. Imaging* **17**, 31–40 (2006), DOI: 10.1097/01.rmr.0000245459.82782.e4.

42. Hernández-Torres, E., Wiggermann, V., Machan, L. *et al.* Increased mean R2* in the deep gray matter of multiple sclerosis patients: Have we been measuring atrophy? *J. Magn. Reson. Imaging* **50**, 201–208 (2019), DOI: 10.1002/jmri.26561.

43. Fields, R. D. White matter in learning, cognition and psychiatric disorders. *Trends Neurosci.* **31**, 361–370 (2008), DOI: 10.1016/j.tins.2008.04.001.

44. Buyanova, I. S. & Arsalidou, M. Cerebral White Matter Myelination and Relations to Age, Gender, and Cognition: A Selective Review. *Front. Hum. Neurosci.* **15**, 1–22 (2021), DOI: 10.3389/fnhum.2021.662031.

45. Stern, Y. Arenaza-Urquijo, E. M., Bartrés-Faz, D. *et al.* Whitepaper: Defining and investigating cognitive reserve, brain reserve, and brain maintenance. *Alzheimer's Dement.* **16**, 1305–1311 (2020), DOI: 10.1016/j.jalz.2018.07.219 .

46. Galland-Decker, C., Marques-Vidal, P. & Vollenweider, P. Prevalence and factors associated with fatigue in the Lausanne middle-aged population: A population-based, cross-sectional survey. *BMJ Open* **9**, 1–10 (2019), DOI: 10.1136/bmjopen-2018-027070.

47. Penner, I.K. & Paul, F. Fatigue as a symptom or comorbidity of neurological diseases. *Nat Rev Neurol*. **13**, 662-675 (2017). doi: 10.1038/nrneurol.2017.117.

48. Tardy, A.L., Pouteau, E., Marquez, D., Yilmaz, C., & Scholey, A. Vitamins and Minerals for Energy, Fatigue and Cognition: A Narrative Review of the Biochemical and Clinical Evidence. Nutrients **16,** 228 (2020), DOI: 10.3390/nu12010228.

49. Haß, U, Herpich, C. & Norman, K. Anti-Inflammatory Diets and Fatigue. Nutrients **30**, 2315 (2019), DOI: 10.3390/nu111102315.

50. Pelletier, A., Barul, C., Féart, C., et al.. Mediterranean diet and preserved brain structural connectivity in older subjects. Alzheimers Dement. **11**, 1023-31 (2015), DOI: 10.1016/j.jalz.2015.06.1888.

51. Torres-Velázquez, M., Sawin, E.A., Anderson, J.M. & Yu, J.J.. Refractory diet-dependent changes in neural microstructure: Implications for microstructural endophenotypes of neurologic and psychiatric disease. Magn Reson Imaging. **58**, 148-155 (2019), DOI: 10.1016/j.mri.2019.02.006.

52. Hechenberger, S., Helmlinger, B., Penner, I.K., et al. Psychological factors and brain magnetic resonance imaging metrics associated with fatigue in persons with multiple sclerosis. J Neurol Sci. **15**, 120833 (2023), DOI: 10.1016/j.jns.2023.120833.

53. Tarasiuk, J., Kapica-Topczewska, K., Czarnowska, A., Chorązy, M., Kochanowicz, J., Kułakowska,, A. Co-occurrence of fatigue and depression in people with multiple sclerosis: a mini-review, Front. Neurol. **12,** 1–8 (2022), DOI: 10.3389/fneur.2021.817256

54. Palotai, M., Cavallari, M., Koubiyr, I. et al. Microstructural fronto-striatal and temporo-insular alterations are associated with fatigue in patients with multiple sclerosis independent of white matter lesion load and depression. Mult Scler. **26**, 1708-1718 (2020), DOI: 10.1177/1352458519869185.

55. Biasi, M.M., Manni, A., Pepe, I. *et al*. Impact of depression on the perception of fatigue and information processing speed in a cohort of multiple sclerosis patients. *BMC Psychol 11*, **208** (2023). DOI: 10.1186/s40359-023-01235-x

56. Bagnato, F., Hametner, S., Boyd, E. *et al.* Untangling the R2* contrast in multiple sclerosis: A combined MRI-histology study at 7.0 Tesla. *PLoS One* **13**, (2018), DOI: 10.1371/journal.pone.0193839.

57. Hametner, S., Endmayr, V., Deistung, A. *et al.* The influence of brain iron and myelin on magnetic susceptibility and effective transverse relaxation - A biochemical and histological validation study. *Neuroimage* **179**, 117–133 (2018), DOI: 10.1016/j.neuroimage.2018.06.007 .

58. Stüber, C., Morawski, M., Schäfer, A. *et al.* Myelin and iron concentration in the human brain: A quantitative study of MRI contrast. *Neuroimage* **93**, 95–106 (2014), DOI: 10.1016/j.neuroimage.2014.02.026.

59. Stankiewicz, J. M., Neema, M. & Ceccarelli, A. Iron and multiple sclerosis. *Neurobiology of Aging* **35**, (2014), DOI: 10.1016/j.neurobiolaging.2014.03.039 .

60. Capone, F., Collorone, S., Cortese, R., Di Lazzaro, V. & Moccia, M. Fatigue in multiple sclerosis: The role of thalamus. *Mult. Scler. J.* **26**, 6–16 (2020), DOI: 10.1177/1352458519851247 .