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Romano, F

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ABNORMAL THALAMIC FUNCTIONAL CONNECTIVITY CORRELATES WITH CARDIORESPIRATORY FITNESS AND PHYSICAL ACTIVITY IN PROGRESSIVE MULTIPLE SCLEROSIS

¹Francesco Romanò, ²Robert W. Motl, ¹Paola Valsasina, ^{3,4}Maria Pia Amato, ^{5,6}Giampaolo Bricchetto, ⁷Nicolò Bruschi, ⁸Jeremy Chataway, ^{9,10}Nancy D. Chiaravalloti, ¹¹Gary Cutter, ¹²Ulrik Dalgas, ^{9,10}John DeLuca, ⁸Rachel Farrell, ^{13,14}Peter Feys, ¹⁵Jennifer Freeman, ^{7,16}Matilde Inglese, ¹⁷Cecilia Meza, ¹⁸Amber Salter, ^{9,10}Brian M. Sandroff, ¹⁷Anthony Feinstein, ^{1,19,20}Maria A. Rocca, ^{1,19,20,21,22}Massimo Filippi, CogEx Research Team.

1. Neuroimaging Research Unit, Division of Neuroscience, IRCCS San Raffaele Scientific Institute, Via Olgettina 60, 20132 Milan, Italy; 2. Department of Kinesiology and Nutrition, University of Illinois Chicago, Chicago, IL, USA; 3. Department NEUROFARBA, Section Neurosciences, University of Florence, Italy; 4. IRCCS Fondazione Don Carlo Gnocchi, Florence, Italy; 5. Scientific Research Area, Italian Multiple Sclerosis Foundation (FISM), via Operai 40, 16149, Genoa, Italy; 6. AISM Rehabilitation Service, Italian Multiple Sclerosis Society, Via Operai 30, 16149, Genoa, Italy; 7. Department of Neuroscience, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health, and Center of Excellence for Biomedical Research, University of Genoa, Genoa, Italy; 8. Queen Square MS Centre, UCL Queen Square Institute of Neurology, Faculty of Brain Sciences, UCL, London, UK; 9. Kessler Foundation, West Orange, NJ, USA; 10. Department of Physical Medicine & Rehabilitation, Rutgers NJ Medical School, Newark, NJ, USA; 11. Department of Biostatistics, University of Alabama at Birmingham, Birmingham, AL, USA; 12. Exercise Biology, Department of Public Health, Aarhus University, Dalgas Avenue 4, DK-8000 Aarhus, Denmark; 13. REVAL, Faculty of Rehabilitation Sciences, Hasselt University, Diepenbeek, Belgium; 14. UMSC Hasselt, Pelt, Belgium; 15. Faculty of Health, School of Health Professions, University of Plymouth, Devon, UK; 16. IRCCS Ospedale Policlinico San Martino, Genoa, Italy; 17. Department of Psychiatry, University of Toronto and Sunnybrook Health Sciences Centre, Toronto, ON M5R 3B6, Canada; 18. Department of Neurology, Section on Statistical Planning and Analysis, UT Southwestern Medical Center, Dallas, TX, USA; 19. Neurology Unit, IRCCS San Raffaele Scientific Institute, Milan, Italy; 20. Vita-Salute San Raffaele University, Milan, Italy; 21. Neurorehabilitation Unit, IRCCS San Raffaele Scientific Institute, Milan, Italy; 22. Neurophysiology Service, IRCCS San Raffaele Scientific Institute, Milan, Italy.

Correspondence should be addressed to: Prof. Massimo Filippi. Full address: Neuroimaging Research Unit, Division of Neuroscience, IRCCS San Raffaele Scientific Institute and Vita-Salute San Raffaele University, 20132 Milan, Italy. Telephone number: +39-02-26433033; Fax number: +39-02-26433031. E-mail address: filippi.massimo@hsr.it

ORCID IDs

John DeLuca: 0000-0002-2767-3382.

Peter Feys: 0000-0002-5680-5495.

Jennifer Freeman: 0000-0002-4072-9758.

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ABSTRACT

Background: Altered thalamic volumes and resting state (RS) functional connectivity (FC) might be associated with physical activity (PA) and cardiorespiratory fitness (CRF) in people with progressive multiple sclerosis (PMS).

Objectives: To assess thalamic structural and functional alterations and investigate their correlations with PA/CRF levels in people with PMS.

Methods: Seven-day accelerometry and cardiopulmonary exercise testing were used to assess PA/CRF levels in 91 persons with PMS. They underwent 3.0T structural and RS fMRI acquisition with 37 age/sex-matched healthy controls (HC). Between-group comparisons of MRI measures and their correlations with PA/CRF variables were assessed.

Results: PMS people had lower volumes compared to HC (all $p < 0.001$). At corrected threshold, PMS showed decreased intra- and inter-thalamic RS FC, and increased RS FC between the thalamus and the hippocampus, bilaterally. At uncorrected threshold, decreased thalamic RS FC with caudate nucleus, cerebellum and anterior cingulate cortex (ACC), as well as increased thalamic RS FC with occipital regions, were also detected. Lower CRF, measured as peak oxygen consumption (VO_{2peak}), correlated with lower white matter volume ($r = 0.31$, $p = 0.03$). Moreover, lower levels of light PA correlated with increased thalamic RS FC with the right hippocampus ($r = -0.3$, $p = 0.05$).

Discussion: People with PMS showed widespread brain atrophy, as well as pronounced intra-thalamic and thalamo-hippocampal RS FC abnormalities. White matter atrophy correlated with CRF, while increased thalamo-hippocampal RS FC was associated to worse PA levels. Thalamic RS FC might be used to monitor physical impairment and efficacy of rehabilitative and disease-modifying treatments in future studies.

INTRODUCTION

Multiple sclerosis (MS) is a chronic, immune-mediated disease of the central nervous system¹ characterized by acute relapses, neurodegeneration, and disability progression.² The progression of disability is often driven by sensory, cerebellar and motor dysfunction, and worsens in severity among persons with progressive (P) compared with relapsing-remitting (RR) MS. The progression of disability and worsening severity of MS often erode quality of life.³ There has been increasing interest in rehabilitation, particularly participation in physical activity, as an approach for managing PMS, as there are few effective pharmacological interventions for this subset of MS.⁴

There is increasing evidence that persons with MS have lower levels of cardiorespiratory fitness (CRF) and physical activity (PA) compared with healthy controls (HC).^{5,6} This is alarming as low levels of PA/CRF have been associated with higher relapse rate, worse disability, larger risk of comorbid conditions, and future clinical worsening.^{7,8} Possible mechanisms underlying these findings might be the neuroprotective effects of physical exercise, such as modulating the concentration of markers of neuroinflammation and improving blood-brain barrier function⁹. Of note, PA/CRF, considered as cross-sectional surrogate outcomes for exercise, have been associated with magnetic resonance imaging (MRI) measures in people with MS. Better CRF was correlated with higher gray matter (GM) volumes and preserved white matter (WM) microstructural integrity,¹⁰ as well as with higher volumes of subcortical GM structures in MS.¹¹ Moderate-to-vigorous PA (MVPA) has similarly been associated with GM and WM in MS.¹² One recent study highlighted an association between MVPA and volumes of the hippocampus and thalamus.¹³ Importantly, these studies enrolled mostly RRMS patients and explored only structural MRI variables, yet there is some recent evidence that the associations among PA, CRF, and cognition as a surrogate of CNS status might not replicate in PMS.¹⁴ To that end, it is worthwhile to study MRI correlates of PA/CRF in patients with PMS, who are characterized by more severe clinical disability and a large range of impaired functions.¹⁵ Such research should further investigate functional MRI

(fMRI) measures when examining the relationship between PA/CRF status and markers of CNS status in MS.

Resting state (RS) fMRI has many advantages for studying PA/CRF and brain plasticity in PMS. RS fMRI avoids confounding effects based on a subject's difficulty when complying with a task: therefore, it is particularly valuable to study the sensorimotor system in people with PMS, who are characterized by moderate-to-severe disability, and are impaired or unable to perform even simple motor tasks during an active fMRI scanning.¹⁶ One strong candidate region for RS functional connectivity (FC) analysis is the thalamus, which is a central deep GM structure having a role in motor planning, sensory information processing and cognitive functions. The presence of extensive thalamic damage has been well documented in MS.¹⁷ Moreover, complex thalamic RS FC alterations have been described across all disease stages, with MS people exhibiting increased¹⁸⁻²² or decreased^{16, 23, 24} intra- and inter-thalamic RS FC, as well as mixed patterns of increased and decreased RS FC between the thalamus and frontal, parietal, temporal and cerebellar cortices.^{16, 18-26} Nevertheless, there is a consistent association of altered thalamic RS FC with clinical disability,^{16, 19, 25} motor¹⁸ and cognitive impairment,^{16, 21, 23, 24} structural damage²² and other clinical symptoms such as fatigue.²⁶ Given the extensive involvement of the thalamus in MS, its key role of integrating multisensory information from several afferent pathways to produce complex motor outputs could be central in explaining exercise-related neuroplasticity. Hence, we hypothesize that altered RS FC of thalamic network might be associated with PA/CRF variables in PMS, and that thalamic RS FC abnormalities might help to better understand adaptive and maladaptive mechanisms associated with a PA/CRF status in PMS. Given that PA measures reflect a behavior which can be influenced by multiple factors, while CRF is a physiological metric of exercise capacity, we expect more substantial correlations with the latter variable.

The current study: i) assessed thalamic damage and RS FC alterations in the thalamic network in people with PMS; and ii) investigated correlations between PA/CRF levels and thalamic structural/functional alterations in these individuals.

METHODS

Study design. This is a cross-sectional study, based on baseline data collected between March 2019 and March 2022 from a multicenter, randomized, sham-controlled trial called “Improving Cognition in People With Progressive Multiple Sclerosis Using Aerobic Exercise and Cognitive Rehabilitation” (CogEx, identifier number: NCT03679468).²⁷ Only subjects recruited in the four centers participating to the MRI sub-study were included in the analyses: a) IRCCS San Raffaele Hospital (Milan, Italy); b) University of Genoa (Genoa, Italy); c) University of Alabama (Birmingham, Alabama, USA) and d) Kessler Foundation (East Hanover, New Jersey, USA). All procedures described below were standardized across sites via comprehensive in-person and remote training, along with quality control on a case-by-case basis.

Participants. Detailed inclusion/exclusion criteria for the MS sample have been previously described.²⁷ Briefly, inclusion criteria were: 1) a confirmed diagnosis of PMS;²⁸ 2) aged between 25 and 65 years old; 3) corrected visual acuity >20/70; 4) intact language comprehension based on Token Test scores >28 (Italian for the centers of Milan and Genoa, English for the centers of Birmingham and East Hanover) and ability to understand instructions; 5) insufficiently active based on a Health Contribution Score of the Godin Leisure-Time Exercise Questionnaire <23 units;²⁹ 6) not severely depressed based on the Beck Depression Inventory-II scores <29; 7) impaired cognitive processing speed based on Symbol Digit Modalities Test (SDMT) scores ≥ 1.282 standard deviation-units below the age-, sex-, and education-adjusted normative score (i.e., ≤ 10 th percentile).³⁰ Exclusion criteria were: 1) wheelchair dependency (Expanded Disability Status Scale [EDSS] score ≥ 7.0); 2) history of significant neurological or psychiatric conditions other than PMS; 3) relapses or steroid use within the past 3 months; 4) MRI contraindications (e.g., pacemaker, pregnancy, breast-feeding, etc.); 5) use of drugs that could affect cognition (excluding cannabis).

We included a sample of 37 HC, without neurologic diseases or systemic disorders, selected for being matched for age ($p=0.16$, two sample t test) and sex ($p=0.84$, Fisher’s exact test) with the

MS group. The sample was included from the research database of IRCCS San Raffaele Hospital and served as reference for structural and functional MRI findings.

Clinical assessment. Within three days from MRI acquisition, people with MS underwent a neurological examination, performed by experienced neurologists blinded to MRI findings, with EDSS score rating³¹ and recording of disease-modifying treatment (DMT). To further characterize the ambulatory status of patients included, the 6-minute walking test (6MWT) was assessed as a measure of walking capacity. It was administered using a standardized protocol³² and the total distance walked was recorded along with the walking aid utilized for the test.

The detailed protocols for the measurements of CRF and PA are reported in a previous publication.¹⁴ Briefly, CRF was measured as peak oxygen consumption ($VO_{2\text{peak}}$) and peak work rate (WR_{peak}) using an incremental cardiopulmonary exercise test (CPET) on a recumbent stepper (NuStep T5XR, NuStep, Inc., Ann Arbor, MI, USA) and an open-circuit spirometry system for analyzing expired gases. $VO_{2\text{peak}}$ was based on the highest recorded 20-second VO_2 value, whereas WR_{peak} was based on the WR recorded at test termination. This testing protocol has been shown to be highly reliable both in patients with moderate (Intraclass Correlation Coefficient [ICC] 0.88) and severe disability (ICC 0.91).³³

PA was measured using waist-worn ActiGraph model GT3X+ accelerometers (ActiGraph, Inc., Pensacola, FL, USA) over a 7-day period. The accelerometer outcomes used for the analyses were average minutes/day of MVPA, light (L) PA, and sedentary behavior.

MRI acquisition. Using 3.0 Tesla scanners (IRCCS San Raffaele: Philips Ingenia CX; University of Genoa and University of Alabama: Siemens Prisma; Kessler Foundation: Siemens Skyra) and standardized procedures for subjects positioning and quality control,²⁷ the following brain MRI sequences were acquired from all subjects during a single session: a) axial T2*-weighted single-shot echo planar imaging (EPI) for RS fMRI (all scanners: repetition time [TR]=1560 ms; echo time [TE]=35 ms, flip angle=70°; multi-band factor=2, matrix size=96×96; field of view [FOV]=240 x 240 mm²; 48 contiguous axial slices, 3 mm thick, number of volumes=320); b)

variable flip angle 3D T2-weighted fluid-attenuated inversion recovery (FLAIR) (Philips: TR=4800 ms; TE=270 ms; inversion time [TI]=1650 ms; matrix size=256 × 256; FOV=256 × 256 mm²; echo train length [ETL]=167; 192 contiguous sagittal slices, 1 mm thick; Siemens: TR=5000 ms; TE=395 ms; TI=1800 ms; matrix size=256 × 256; FOV=256 × 256 mm²; ETL=284; 192 contiguous sagittal slices, 1.05 mm thick), and c) sagittal 3D T1-weighted sequence: (Philips: TR=7 ms; TE=3.2 ms; TI=1000 ms; flip angle=8°; matrix size=256 × 256; FOV=256 × 256 mm²; 204 contiguous sagittal slices, 1 mm thick; Siemens: TR=2300 ms; TE=2.98 ms; TI=900 ms; flip angle=9°; matrix size=256 × 256; FOV=256 × 256 mm²; 204 contiguous sagittal slices, 1 mm thick). Acquisition for RS fMRI scans required about 8 minutes. During RS fMRI acquisition, subjects were asked to keep their eyes closed, to remain motionless and not to think of anything in particular. The total duration of MRI acquisition was approximately 30 minutes.

Structural MRI analysis. Focal T2-hyperintense lesions were identified by a fully automated and validated approach using the 3D FLAIR and 3D T1-weighted as input images³⁴ and T2-hyperintense lesion volume (LV) was derived. Normalized brain volume (NBV), normalized cortical GM volume (NCGMV) and normalized WM volume (NWMV) were measured using FSL SIENAx software on the lesion-filled 3D T1-weighted sequence.³⁵ Segmentation of subcortical GM structures was performed using the FSL FIRST tool; their volume was calculated and normalized using FSL SIENAx scaling factor. The following values were used for subsequent statistical analysis: left and right normalized thalamic volume, left and right normalized hippocampal volumes, and left and right normalized volumes of other deep GM nuclei (NDGMV, i.e., the sum of caudate nucleus, pallidum, putamen, amygdala and nucleus accumbens).

RS FC analysis. RS fMRI data were pre-processed using the CONN toolbox (<https://web.conn-toolbox.org/>).³⁶ RS fMRI images were realigned to the mean of each session with a six-degree rigid-body transformation to correct for minor head movements. After rigid registration of realigned images to the lesion filled 3D T1-weighted scan, RS fMRI images were normalized to the MNI template using a standard affine transformation followed by non-linear warping. After

detection of outliers (using the ART toolbox), images were smoothed with a 6 mm^3 Gaussian filter. The five principal components derived from WM and cerebrospinal fluid (CSF) estimated with the anatomical component-based noise correction method (aCompCor),³⁷ and motion parameters with their first temporal derivatives were regressed out from RS fMRI time series as nuisance covariates. Outliers detected by the ART toolbox (if any) and spurious effects from the first two timepoints (influenced by non steady-state signal) were also regressed out from data. Finally, images were linearly detrended and band-pass filtered (0.01-0.1 Hz).

After pre-processing, RS FC analysis was performed with a seed-based correlation approach using the REST software (www.nitrc.org/projects/rest). Masks of the left and right thalami of each participant, extracted from FSL FIRST, were co-registered to the MNI space by using as reference the normalized 3D T1-weighted scan (i.e., the same image used as reference to normalize RS fMRI scans), and used to separately identify left and right thalamic connectivity maps. RS FC was investigated by calculating the correlation coefficients between the time series separately extracted from both seed regions and any other voxel in the brain. A Fisher's z transform was used to improve the gaussianity of the obtained correlation coefficients.

Statistical analysis. Categorical variables were reported as frequency (%), continuous variables as mean (standard deviation [SD]) or median (interquartile range [IQR]), depending if the variable had a normal or non-normal distribution, respectively. Between-group comparisons of log-transformed T2 LV and brain volumetric measures were assessed using age-, sex-, and site-adjusted ANOVA models (SPSS 26.0 software, IBM corp, Armonk, NY, USA).

RS FC differences between HC and people with MS were analyzed at voxel-wise level using SPM12 and age-, sex- and site-adjusted two-sample t tests. Group differences were assessed within the family-wise error (FWE) corrected mask of effects of interest, in order to investigate RS FC abnormalities only for voxels having a significant connection with left and right thalamic seed regions. To control for the presence of false positives, a minimum cluster extent threshold for between-group comparisons was calculated using the AFNI program 3dClustSim, using as search

masks the effects of interests of left and right thalamic RS FC, respectively.³⁸ Such a calculation gave us as a minimum extent for cluster significance $k=69$ voxels for the left thalamic network, and $k=55$ voxels for the right thalamic network. Due to the exploratory nature of this analysis, significant between-group differences at the uncorrected threshold of $p<0.001$ and cluster extent $k=10$ voxels were also reported. Given the unbalanced design of the study, with HC acquired just at one enrolling site, we performed a sensitivity analysis by repeating the between-group comparison of RS FC differences without adjusting SPM12 models for site.

The mean RS FC Z-scores of regions that showed significant differences and survived the threshold determined with 3dClustSim in the previous analysis were extracted using the REX toolbox (<https://www.nitrc.org/projects/rex>) and used for SPSS correlation analysis, which was performed in PMS only, since no measures of cardiorespiratory fitness and PA were available for HC. More specifically, correlations between clinical variables (i.e., EDSS score, disease duration) and behavior/fitness variables (i.e., MVPA, LPA, sedentary behavior, VO_{2peak} , and WR_{peak}) with MRI measures were assessed using partial correlations corrected for age and sex (SPSS 26.0 software). Adjusted Spearman rank-order correlation coefficients with the respective p values were reported. A statistical significance level of $p<0.05$ was used, false discovery rate (FDR) correction according to Benjamini-Hochberg was applied to control for multiple comparisons.³⁹

RESULTS

Demographic, clinical and structural MRI findings. Demographic, clinical, and structural MRI variables of HC and people with PMS are reported in Table 1.

There were 128 subjects included in the study, of which 91 were people with PMS (58 females; mean age=51.7 years [SD=6.97]) with a median EDSS of 6.0 (IQR=4.0-6.5) and median disease duration of 15 years (IQR=7-24), and 37 HC (25 females; mean age=49.4 years [SD=10.64]).

The measures of PA were not available for 9 MS people, based on the wear-time not meeting minimal thresholds for inclusion as a valid day of data. Therefore, these 9 MS people were only used for between-group MRI comparisons and to analyze correlations with CRF.

People with PMS had higher T2 LV ($p < 0.001$) and lower NBV, NCGMV, NWMV, thalamic, hippocampal, and NDGMV ($p < 0.001$ for all comparisons) compared with HC.

Between-group comparison of thalamic RS FC. Results of RS FC analysis are summarized in Table 2 and Figure 1.

At cluster-size corrected threshold, people with PMS had decreased RS FC between the left thalamus and the bilateral thalami and increased RS FC between the left thalamus and the left hippocampus compared with HC. In addition, small uncorrected clusters of decreased RS FC were found in PMS between the left thalamus and left caudate nucleus, bilateral cerebellum (crus II), and bilateral anterior cingulate cortex (ACC), while increased RS FC was found with the right hippocampus.

There was a similar pattern of RS FC alterations in the right thalamic network. At cluster-size corrected threshold, people with PMS had decreased RS FC between the right thalamus and the bilateral thalami, as well as increased RS FC between the right thalamus and the left and right hippocampus. In addition, uncorrected clusters of decreased RS FC were found between the right thalamus and left caudate nucleus and left ACC, while increased RS FC with the left calcarine cortex and bilateral lingual gyrus was found in PMS compared with HC.

The sensitivity analysis indicated that the same pattern of RS FC abnormalities in PMS compared to HC, both for the left and the right thalamic networks, could be detected by models not including site as confounding covariate (data not shown), suggesting that acquisition site did not have a major impact on our findings.

Correlation analysis. No significant correlations were found between structural MRI metrics and PA measures or EDSS score ($p = \text{range } 0.59; 0.99$). Longer disease duration correlated with

lower bilateral NDGMV (r =range -0.33;-0.26, p =range 0.02;0.05, Table 3). Lower VO_{2peak} correlated with lower NWMV (r =0.31, p =0.03, Table 3).

Significant correlations between altered thalamic RS FC and LPA, CRF and clinical measures are reported in Table 4.

No significant correlations were observed between altered thalamic RS FC and EDSS score (p =range 0.67;0.88). Longer disease duration correlated with increased RS FC of the left thalamus with the left hippocampus (r =0.27, p =0.05).

Lower LPA correlated with increased RS FC between the right thalamus and right hippocampus (r =-0.3, p =0.05). No significant correlations were observed between altered RS FC and the remaining measures of LPA or CRF (p =range 0.1;0.84).

DISCUSSION

Herein, we included baseline clinical and MRI data collected from the CogEx trial and assessed correlations between PA/CRF and thalamic structural and functional MRI alterations in people with PMS. Our results indicated the presence of structural and functional thalamic abnormalities in people with PMS. Among all structural MRI measures, reduced WM volume correlated with worse CRF. In addition, altered thalamo-hippocampal RS FC displayed a maladaptive association with lower PA. This suggests that functional MRI changes of the thalamus might be relevant when examining behavioral interventions focused on increasing PA as a rehabilitation avenue for neuroplasticity in people with PMS and moderate-to-severe disability.

The sample of people with PMS considered for this cross-sectional analysis had moderate-to-severe levels of disability; also, 40% of these patients were using a unilateral or bilateral walking aid. Although there were no clinical data available for comparison in the HC group, we found overall low levels of PA and CRF, which are consistent with the results of previous studies that assessed these outcomes in samples of patients with similar characteristics.^{29, 33}

The comparison of structural MRI metrics between people with PMS and HC indicated widespread atrophy of all brain tissue compartments and deep GM nuclei. This is an expected result, as several studies have reported that people with PMS are characterized by severe brain atrophy, both at cortical and subcortical level.¹⁸ This is likely the result of a severe ongoing neurodegeneration as a crucial component of PMS pathophysiology.

Regarding RS fMRI findings, our results indicated the presence of a consistent pattern of bilateral thalamic RS FC abnormalities. We observed the concomitant presence of decreased RS FC within- and between thalami as well as increased thalamo-hippocampal RS FC. The notion that heterogeneous thalamic RS FC alterations occur in MS is not novel, as most previous investigations reported the contemporary presence of increased and decreased thalamic RS FC in the same patient groups.^{16, 18-20, 22, 24, 25} An interesting hypothesis supporting such heterogeneity was recently formulated through a simulation study⁴⁰ wherein thalamic and GM atrophy mainly drive increased thalamic RS FC, while severe WM damage (likely to be present in our PMS group) mainly drives decreased thalamic RS FC.

In line with some studies,^{16, 23, 24} but not with others,^{20, 21} we observed decreased intra- and inter-thalamic RS FC in PMS compared with HC. The discrepancy with previous studies might be based on differences in the enrolled phenotypes²⁰ and/or complex structure of the thalamus, which might demonstrate different RS FC profiles across sub-regions.^{18, 19, 26} On the other hand, an increase in RS FC was present within the thalamic network in PMS people vs HC when considering the bilateral hippocampus. Such increases in thalamo-hippocampal RS FC have been reported in MS, especially in those with poor cognitive performances,^{18, 21, 24} suggesting a maladaptive role of such heightened connectivity between these structures, which reflects less efficient network processing. This is also consistent with the increased thalamo-hippocampal FC observed in older adults with impaired visuospatial memory, compared to cognitively preserved older adults and to young adults.⁴¹ We note that, in line with this concept, all patients included in the CogEx trial were screened for impaired cognitive processing speed.²⁷

Our analysis indicated limited associations between PA/CRF with structural MRI outcomes: significant associations were only reported between lower CRF, measured with VO_{2peak} , and lower NWMV. This finding is consistent with a recent systematic review and meta-analysis, which reported associations of lower WM volume/WM microstructural integrity with lower PA measures in healthy older adults;⁴² this could be explained by reduced protective neurotrophic factors or decreased brain perfusion. Similar pathophysiological processes might be involved in MS.⁴³⁻⁴⁵ Nevertheless, we observed no associations between GM volumes, in particular thalamic volumes, and CRF and MVPA that have been reported in non-PMS.^{10, 11, 12, 13} This novel result might be explained by our patients having confirmed PMS, higher disability, and lower levels of PA and CRF than those enrolled in previous studies. This might create a truncated range of scores for examining the relationship between GM atrophy and PA/CRF in our data. It could also be that neurodegeneration in people with PMS is so vast that the capability to maintain an active lifestyle and GM volumes become unrelated. In any case, the results from the longitudinal analyses of the CogEx study will be able to address these questions.

By comparison, the analysis of RS fMRI data revealed a correlation between PA and thalamic RS FC abnormalities in PMS. Specifically, we observed that lower LPA was associated with increased thalamo-hippocampal RS FC. PA (and CRF) measures have already been described as important correlates of thalamic and hippocampal volumetrics in people with MS.¹¹⁻¹³ There is convincing evidence that both these structures are important nodes of exercise-related functional plasticity in healthy adults.⁴⁶ However, while hippocampal RS FC has been often put in relationship with memory impairment^{47, 48} and depression,⁴⁹ little is known about its relationship with fitness status in MS. Here, in line with previous memory studies,^{47, 48} increased hippocampal-thalamic RS FC was maladaptive, since it was correlated with poorer LPA performances. The notion that, in MS, RS FC is increased between the thalamus and hippocampus is not novel, and has previously been interpreted as a consequence of RS FC loss between the thalamus and cortical lobes.²⁰ While some clusters of reduced thalamo-cortical RS FC were observed also in this study, no clear conclusions

regarding this point can be drawn due to their limited size. Behavioral interventions aimed at reducing sedentary time and increasing bouts of PA during the day, which have been suggested as promising avenues in populations with high disability and pronounced motor and cognitive deficits,⁵⁰ might be able to modulate thalamo-hippocampal FC, which in turn, is closely tied to cognitive functions.^{18, 24, 41} Of note, there is preliminary evidence that aerobic training resulted in increased thalamo-frontal connectivity, correlating also with improvements in CRF and cognitive processing speed in a small sample of people with RRMS and mild disability.⁵¹

Overall, the magnitude of correlation coefficients observed in this study could be considered in the weak range, as most were comprised between ± 0.2 and ± 0.3 . This, however, does not affect the validity or results obtained here, since works that also assessed the association between thalamic RS FC and clinical measures also noted similar strength of correlations with cognition,^{16, 21} motor performance¹⁸ and fatigue.²⁶

We initially hypothesized that measures of CRF, rather than PA, might show more sizeable correlations with MRI alterations. Given the limited amount of correlations found in our analysis, it is not possible to draw clear conclusions about this point. Future studies addressing additional factors that possibly explain variability in PA and CRF should help elucidate this issue.

Contrary to previous studies,^{18, 25} no correlations were observed between thalamic structural/functional MRI measures and clinical disability, as assessed by the EDSS score. Once again, this negative result might be related to the specific characteristics of our sample: the vast majority of included patients had an EDSS score comprised between 4.0 and 6.5, a relatively narrow range, as a consequence of the strict inclusion criteria of the original randomized controlled trial.²⁷ The lack of variability in the EDSS score might prevent the generation of significant correlations.⁵⁶

This study is not without limitations. First, the CogEx trial²⁷ was not designed to include HC. As such, we had to retrospectively select an independent cohort of HC to serve as reference for structural and functional MRI variables, but unfortunately, no PA/CRF data were available for this

HC cohort. Therefore, we could not investigate whether thalamic volumes and thalamic RS FC were associated with PA and CRF variables in healthy subjects. Also, the lack of PA/CRF data in healthy controls did not allow us to assess correlations within the same full factorial models used for between-group differences. We had to extract mean values from clusters showing altered thalamic RS FC and used such values for *post-hoc* correlation analysis, which is not an optimal strategy because of an intrinsic risk of circularity.⁵⁷ As a consequence, the results of this analysis should be interpreted with caution and they should be first replicated in other samples before possible generalization. Second, we analyzed RS FC of the thalamus considering it as a whole, while it might be possible that different thalamic subregions have different RS FC profiles, as reported in previous investigations.^{18, 19, 26} Future studies might further investigate correlations of sub-regional thalamic RS FC with PA/CRF status in people with MS. Additionally, advanced structural MRI techniques (such as diffusion-weighted MRI) were not included; therefore, we did not assess the relative contribution of microstructural thalamic injury in explaining patients' PA and CRF status. Last, it must be taken into account that the inclusion criteria of impaired cognitive processing speed and reduced self-reported PA, essential prerequisites for participation in the RCT, narrow down the possibility to generalize these results to a broader group of people with MS.

To conclude, we observed widespread structural and functional thalamic abnormalities in people with PMS. While WM atrophy correlated with CRF, abnormal RS FC in the thalamic network was associated with LPA in PMS. Given its clinical eloquence, thalamic RS FC might be an outcome to consider when monitoring physical impairment and efficacy of rehabilitative and disease-modifying treatments in PMS patients in future studies.

Statements and Declarations

Ethical approval

The study was approved by local research ethics committees or institutional review boards at enrolling sites. All participants provided written informed consent prior to study participation according to the Declaration of Helsinki.

Disclosures

Francesco Romanò has nothing to disclose; Robert W. Motl has nothing to disclose; Paola Valsasina received speaker honoraria from Biogen Idec; Maria Pia Amato received compensation for consulting services and/or speaking activities from Bayer, Biogen Idec, Merck-Serono, Novartis, Roche, Sanofi Genzyme, and Teva Pharmaceutical Industries; and receives research support from Biogen Idec, Merck-Serono, Roche, Pharmaceutical Industries and Fondazione Italiana Sclerosi Multipla; Giampaolo Bricchetto has been awarded and receives research support from Roche, Fondazione Italiana Sclerosi Multipla, ARSEP, H2020 EU Call; Nicolò Bruschi has nothing to disclose; Jeremy Chataway has received support from the Efficacy and Evaluation (EME) Programme, a Medical Research Council (MRC) and National Institute for Health Research (NIHR) partnership and the Health Technology Assessment (HTA) Programme (NIHR), the UK MS Society, the US National MS Society and the Rosetrees Trust, he is supported in part by the National Institute for Health Research, University College London Hospitals, Biomedical Research Centre, London, UK, he has been a local principal investigator for commercial trials funded by Actelion, Biogen, Novartis and Roche, has received an investigator grant from Novartis, and has taken part in advisory boards/consultancy for Azadyne, Biogen, Celgene, MedDay, Merck and Roche; Nancy D. Chiaravalloti is on an Advisory Board for Akili Interactive and is a member of the Editorial Boards of Multiple Sclerosis Journal and Frontiers in NeuroTrauma; Gary Cutter is a member of Data and Safety Monitoring Boards for Astra-Zeneca, Avexis Pharmaceuticals, Biolinerx, Brainstorm Cell Therapeutics, Bristol Meyers Squibb/Celgene, CSL Behring, Galmed Pharmaceuticals, Horizon Pharmaceuticals, Hisun Pharmaceuticals, Mapi Pharmaceuticals LTD, Merck, Merck/Pfizer, Opko Biologics, OncoImmune, Neurim, Novartis, Ophazyme, Sanofi Aventis, Reata Pharmaceuticals, Teva pharmaceuticals, VielaBio Inc, Vivus, NHLBI (Protocol Review Committee), NICHD (OPRU oversight committee), he is on Consulting or Advisory Boards for Biodelivery Sciences International, Biogen, Click Therapeutics, Genzyme, Genentech, GW Pharmaceuticals, Klein-Buendel Incorporated, Medimmune, Medday, Neurogenesis LTD, Novartis, Osmotica Pharmaceuticals, Perception Neurosciences, Recursion/Cerexis Pharmaceuticals, Roche, TG Therapeutics, he is employed by the University of Alabama at

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Data availability

The anonymised dataset used and analysed during the current study is available from the corresponding author upon reasonable request.

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Table 1. Demographic, clinical, and structural MRI characteristics of healthy controls (HC) and people with multiple sclerosis (MS) enrolled in this study.

	HC n=37	People with MS n=91	p value
Mean age (SD) [years]	49.4 (10.6)	51.7 (6.9)	0.16 [§]
Male/Female (%)	12/25 (32%/68%)	33/58 (36%/64%)	0.84 [°]
Median EDSS (IQR)	-	6.0 (4.0-6.5)	-
Median disease duration (IQR) [years]	-	15 (7-24)	-
DMT (None/1 st line/2 nd line) (%)	-	33/22/36 (36%/24%/40%)	-
Median 6MWT (IQR) [m]	-	226 (120-351)	-
Walking aid			
None (%)	-	55 (60%)	-
Unilateral (%)	-	4 (5%)	-
Bilateral (%)	-	32 (35%)	-
Median VO _{2peak} (IQR) [mL/min/kg]	-	14.2 (11.3-18.5)	-
Median WR _{peak} (IQR) [W]	-	65 (45-90)	-
Median MVPA* (IQR) [min/day]	-	4.6 (1.4-18.8)	-
Median LPA* (IQR) [min/day]	-	249.7 (170.4-306.5)	-
Median sedentary* (IQR) [min/day]	-	502.6 (442.1-587.5)	-
Median T2 LV (IQR) [mL]	0.18 (0.00-0.48)	7.47 (4.21-19.00)	<0.001 ⁺
Mean NBV (SD) [mL]	1542 (39)	1483 (68)	<0.001 ⁺
Mean NcGMV (SD) [mL]	639 (33)	616 (38)	<0.001 ⁺
Mean NWMV (SD) [mL]	691 (26)	666 (41)	<0.001 ⁺
Mean L NThalV (SD) [mL]	11.25 (0.69)	9.81 (1.41)	<0.001 ⁺
Mean R NThalV (SD) [mL]	10.83 (0.64)	9.47 (1.47)	<0.001 ⁺
Mean L NHippV (SD) [mL]	5.47 (0.57)	4.60 (0.81)	<0.001 ⁺
Mean R NHippV (SD) [mL]	5.65 (0.42)	4.74 (0.79)	<0.001 ⁺
Mean L NDGMV (SD) [mL]	16.84 (0.99)	14.45 (2.09)	<0.001 ⁺
Mean R NDGMV (SD) [mL]	16.72 (0.96)	14.65 (1.91)	<0.001 ⁺

[§]two-sample t test; [°] Fisher's exact test; ⁺age-, sex- and site-adjusted ANOVA.

Abbreviations: SD=standard deviation; EDSS=Expanded Disability Status Scale; IQR=interquartile range; DMT=disease-modifying treatment; 6MWT=6-minute walking test; VO_{2peak}=peak oxygen consumption; WR_{peak}=peak work rate; MVPA=moderate-to-vigorous physical activity; LPA=light physical activity; T2 LV=T2 hyperintense lesion volume; NBV=normalized brain volume; NcGMV=normalized cortical gray matter volume; NWMV=normalized white matter volume; NThalV=normalized thalamic volume; NHippV=normalized hippocampal volume;

NDGMV=normalized deep gray matter volume (sum of caudate nucleus, putamen, pallidum, amygdala, and nucleus accumbens); L=left; R=right.

*9 people with MS with missing data.

Table 2. Between-group comparison of RS FC within the left and right thalamic networks (SPM12 two-sample t test between healthy controls [HC] and people with multiple sclerosis [MS], adjusted for age, sex and acquisition site). The minimum cluster extent thresholds (k) being relevant for significance, calculated using the AFNI program 3dClustSim, were 69 and 55 for the left and right thalamic network, respectively. Results surviving at this threshold are marked with *. Uncorrected clusters significant at $p < 0.001$ are also reported.

Network	Comparison	Areas	MNI space coordinates	k	T value
L Thalamus	MS < HC	R Thalamus*	2 -16 6	459	6.18
		L Thalamus*	-12 -18 14		4.04
		R Thalamus	16 -4 10	32	4.12
		L Caudate nucleus	-12 18 10	18	3.76
		R Cerebellum crus II	8 -84 -38	28	4.33
		L Cerebellum crus II	-14 -84 -44	31	4.28
		R ACC	2 36 16	47	3.87
		L ACC	-10 20 24	21	3.70
	MS > HC	L Hippocampus/PHG*	-12 -24 0	155	5.19
		R PHG	22 -32 -10	17	3.97
R Thalamus	MS < HC	R Thalamus*	2 -16 6	450	4.64
		L Thalamus*	-10 -14 16		4.51
		L Caudate nucleus	-8 16 10	12	4.11
		L ACC	-2 22 28	17	3.34
	MS > HC	L PHG*	-28 -22 -18	120	4.91
		R PHG*	24 -30 -14	96	4.12
		L Calcarine cortex	-16 -60 14	10	3.64
		L Lingual gyrus	-14 -52 2	22	3.44
		R Lingual gyrus	18 -52 4	26	3.44

Abbreviations: L=left; R=right; ACC=anterior cingulate cortex; PHG=parahippocampal gyrus.

Table 3. Associations between global and subcortical structural MRI measures and clinical variables in people with multiple sclerosis (MS). Significant correlations (false discovery rate [FDR]-corrected) are indicated in **bold**.

Structural MRI Measure	EDSS r (p value)*	Disease duration r (p value)*	VO ₂ peak r (p value)*	WR _{peak} r (p value)*	MVPA r (p value)*	LPA r (p value)*	Sedentary r (p value)*
T2 LV	0.1 (0.8)	0.18 (0.13)	-0.03 (0.88)	-0.08 (0.83)	-0.14 (0.59)	0.06 (0.87)	-0.05 (0.83)
NBV	-0.1 (0.8)	-0.22 (0.1)	0.14 (0.86)	0.21 (0.25)	0.12 (0.59)	-0.03 (0.87)	0.15 (0.82)
NcGMV	0.002 (0.99)	-0.12 (0.25)	-0.09 (0.86)	0.02 (0.93)	0.15 (0.59)	-0.03 (0.87)	0.1 (0.82)
NWMV	-0.13 (0.8)	-0.21 (0.1)	0.31 (0.03)	0.28 (0.1)	0.08 (0.59)	0.02 (0.87)	0.16 (0.82)
L NThalV	-0.07 (0.8)	-0.2 (0.1)	0.04 (0.86)	0.13 (0.68)	0.04 (0.72)	-0.05 (0.87)	0.07 (0.83)
R NThalV	-0.05 (0.8)	-0.25 (0.07)	-0.1 (0.91)	0.07 (0.83)	0.05 (0.72)	-0.02 (0.87)	0.03 (0.83)
L NHippV	-0.08 (0.8)	-0.19 (0.11)	-0.11 (0.86)	0.003 (0.98)	0.08 (0.59)	0.05 (0.87)	-0.1 (0.82)
R NHippV	-0.001 (0.99)	-0.16 (0.14)	0.05 (0.86)	0.06 (0.83)	0.1 (0.59)	0.03 (0.87)	-0.03 (0.83)
L NDGMV	-0.12 (0.8)	-0.26 (0.05)	0.09 (0.86)	0.12 (0.68)	0.12 (0.59)	-0.16 (0.87)	0.06 (0.83)
R NDGMV	-0.05 (0.8)	-0.33 (0.02)	0.07 (0.86)	0.03 (0.93)	0.1 (0.59)	-0.12 (0.87)	0.1 (0.82)

*Partial correlations adjusted for age, sex, and acquisition center. Correlations are reported as adjusted Spearman rank-order correlation coefficients (r) and corresponding FDR-corrected p values.

Abbreviations: T2 LV=T2 hyperintense lesion volume; NBV=normalized brain volume; NcGMV=normalized cortical gray matter volume;

NWMV=normalized white matter volume; NThalV=normalized thalamic volume; NHippV=normalized hippocampal volume;

NDGMV=normalized deep gray matter volume (sum of caudate nucleus, putamen, pallidum, amygdala, and nucleus accumbens); L=left; R=right.

Table 4. Associations between abnormal resting state (RS) functional connectivity (FC) in thalamic networks and clinical variables in people with multiple sclerosis (MS). Significant correlations (false discovery rate [FDR]-corrected) are indicated in **bold**.

Thalamic network RS FC abnormalities	EDSS r (p value)*	Disease duration r (p value)*	VO_{2peak} r (p value)*	WR_{peak} r (p value)*	MVPA r (p value)*	LPA r (p value)*	Sedentary r (p value)*
L Thalamic network Decreased RS FC in the bilateral thalamus	-0.09 (0.67)	-0.21 (0.08)	0.2 (0.1)	0.17 (0.18)	0.23 (0.13)	0.09 (0.58)	0.16 (0.4)
L Thalamic network Increased RS FC with the L hippocampus/PHG	0.05 (0.85)	0.27 (0.05)	-0.21 (0.1)	-0.09 (0.5)	-0.08 (0.61)	-0.02 (0.89)	-0.08 (0.58)
R Thalamic network Decreased RS FC in the bilateral thalamus	-0.12 (0.67)	-0.21 (0.08)	0.19 (0.1)	0.19 (0.18)	0.22 (0.13)	0.1 (0.58)	0.19 (0.4)
R Thalamic network Increased RS FC with the L hippocampus/PHG	0.02 (0.88)	0.14 (0.25)	-0.07 (0.51)	-0.04 (0.74)	-0.04 (0.76)	-0.22 (0.13)	-0.02 (0.84)
R Thalamic network Increased RS FC with the R hippocampus/PHG	0.14 (0.67)	0.11 (0.3)	-0.19 (0.1)	-0.18 (0.18)	-0.13 (0.4)	-0.3 (0.05)	-0.09 (0.58)

*Partial correlations adjusted for age and sex. Correlations are reported as adjusted Spearman rank-order correlation coefficients (r) and corresponding FDR-corrected p values.

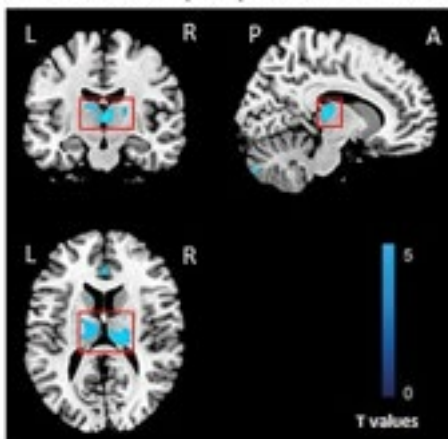
Abbreviations: L=left; R=right; PHG=parahippocampal gyrus; VO_{2peak}=peak oxygen consumption; WR_{peak}=peak work rate; MVPA=moderate-to-vigorous physical activity; LPA=light physical activity.

Figure legends

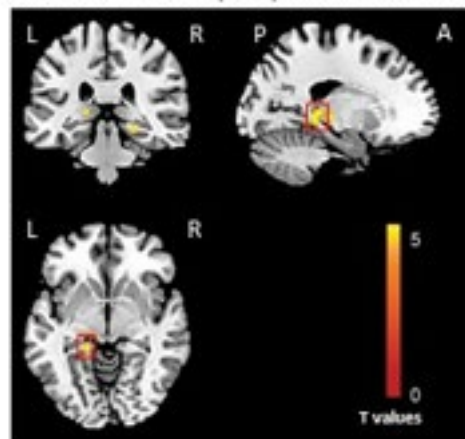
Figure 1. Resting state (RS) functional connectivity (FC) abnormalities in people with multiple sclerosis (MS) compared with healthy controls (HC), obtained using SPM12 and two-sample t tests, adjusted for age, sex and acquisition site.

Results are shown at $p < 0.001$, uncorrected (cluster extent $k = 10$ voxels) for illustrative purposes (decreased RS FC: blue-lightblue, increased RS FC: red-yellow). Clusters surviving the corrected threshold are highlighted within red boxes. Images are in neurological convention.

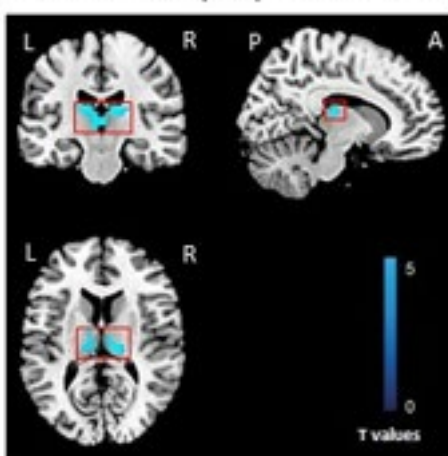
Left Thalamus – people with PMS < HC



Left Thalamus – people with PMS > HC



Right Thalamus – people with PMS < HC



Right Thalamus – people with PMS > HC

