Original Research Paper

Altered anterior default mode network dynamics in progressive multiple sclerosis

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Abstract

Background: Modifications in brain function remain relatively unexplored in progressive multiple sclerosis (PMS), despite their potential to provide new insights into the pathophysiology of the disease at this stage.

Objectives: To characterize the dynamics of functional networks at rest in patients with PMS, and the relation with clinical disability.

Methods: Thirty-two patients with PMS underwent clinical and cognitive assessment. The dynamic properties of functional networks, retrieved from transient brain activity, were obtained from patients and 25 healthy controls (HCs). Sixteen HCs and 19 patients underwent a 1-year follow-up (FU) clinical and imaging assessment. Differences in the dynamic metrics between groups, their longitudinal changes, and the correlation with clinical disability were explored.

Results: PMS patients, compared to HCs, showed a reduced dynamic functional activation of the anterior default mode network (aDMN) and a decrease in its opposite-signed co-activation with the executive control network (ECN), at baseline and FU. Processing speed and visuo-spatial memory negatively correlated to aDMN dynamic activity. The anti-couplings between aDMN and auditory/sensory-motor network, temporal-pole/amygdala, or salience networks were differently associated with separate cognitive domains.

Conclusion: Patients with PMS presented an altered aDMN functional recruitment and anti-correlation with ECN. The aDMN dynamic functional activity and interaction with other networks explained cognitive disability.

Keywords: Progressive multiple sclerosis, resting-state functional magnetic resonance imaging, innovation-driven co-activation patterns, default mode network, clinical disability

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Introduction

Progressive multiple sclerosis (PMS) is characterized by a gradual worsening in clinical disability, occurring from the onset of the disease in the case of primary progressive (PP) form, or after a relapsing-remitting (RR) phase in secondary progressive (SP) multiple sclerosis (MS). PP and SP MS phenotypes are considered different aspects of a unique spectrum[1] and this disease stage, compared to RR MS, presents with a slower, chronic, compartmentalized inflammation and a prominent neurodegeneration, together with a depleted ability to respond to damage. The contribution of imaging to the definition of PMS ranges from the identification of biomarkers, as atrophy, to the detection, in vivo, of hallmarks such as cortical lesions and diffuse white matter damage.[1,2] Nonetheless, while a growing number of reports is revealing the clinical significance of the networks function disruption occurring in MS, studies targeting brain functional activity in PMS are relatively lacking.

Previous studies of brain function at rest in patients with PMS reported altered network activity or connectivity involving the default mode network (DMN),[3] attentional, executive control and sensory-motor (SM) networks.[4–6] However, the discordant results and the limited number of studies do not allow a unique interpretation of the changes in functional networks occurring in this phase of the disease and the hypothesis of an exhaustion of plasticity characterizing PMS is mainly inferred by studies on RR MS patients at later stages. Moreover, to our knowledge, only a longitudinal study focusing on the SM

functional network has been performed, so far.[7] As emerging for other neurodegenerative conditions, defining the functional disruption occurring in PMS could contribute to the understanding of pathophysiology, to extricate the changes due to the disease or to brain aging and to pinpoint potential treatments.[8]

Beside conventional analysis, new approaches have recently emerged to explore the time-varying nature of brain functional activity at rest.[9] In healthy subjects, the investigation of the dynamic properties of resting-state functional connectivity (rs-FC) has revealed its ability in better grasping behavioral aspects, compared to the classic time-averaged connectivity.[10] The dynamics of rs-FC have also been investigated in patients at the earlier stages of MS, emphasizing the complex functional changes occurring in this disorder and their relationship to the cognitive status.[11–16] Moreover, the ability of dynamic rs-FC in better capturing cognitive decline has been showed in neurodegenerative disorders.[17]

Multiple methodologies have been developed to study the variations over time in functional brain activity. Among them, the characterization of patterns of functional co-activation among regions is able to tackle the pathophysiological processes occurring in neurological or neuropsychiatric disorders, and to parallel the clinical improvement after therapeutic procedures.[18–20]

In this study, we hypothesized that the dynamics of functional networks, investigated by means of co-activation patterns, would be altered in patients with PMS and would explain clinical disability. Specifically, we investigated: (1) the dynamics of functional networks in patients with PMS, compared to healthy controls (HCs), (2) their changes after 1 year of follow-up (FU), and (3) their role in explaining cognitive impairment, assessed with the Brief International Cognitive Assessment for Multiple Sclerosis, EDSS and motor disability.

Materials and methods

Participants
Forty-eight patients with either PP or SP MS and 26 HCs were prospectively recruited. Inclusion and exclusion criteria are detailed in the Supplementary Information (SI). After image quality check and motion correction (detailed in SI), the final sample included 57 subjects: 32 patients with PMS (mean age = 50.8 ± 9.9 years, 20 women, 18 patients with PP MS and 14 patients with SP MS) and 25

HCs (mean age = 47.3 ± 8.7 years, 10 women). All subjects underwent magnetic resonance imaging (MRI) and, on the same day, patients underwent clinical evaluation, including Expanded Disability Status Scale (EDSS), timed 25-foot walking (T25FW) test, 9-hole peg test (9HPT), and neuropsychological assessment with the Symbol Digit Modalities Test (SDMT), California Verbal Learning Test–Second Edition (CVLT-II) and the Brief Visuo-spatial Memory Test–Revised (BVMT-R). For the CVLT-II and the BVMT-R, the first five or three recall trials were considered, respectively. Raw scores were transformed into *Z*-scores according to Parmenter et al.[21] Impairment for a single test was assessed on the level of 1.5 standard deviation (SD), and patients were defined as cognitively impaired (CI) or cognitively preserved (CP) based on scoring outside the normal range in one or more of the tests.[22]

Thirty-five (16 HCs and 19 patients, 11 with PP MS and 8 with SP MS) out of the 57 subjects included at baseline underwent MRI and clinical evaluation after 1 year ((mean value ± SD) = 11 ± 2 months). Subjects who were included at FU or dropped out from the study did not differ in terms of demographic, clinical, and imaging variable (see Table SI 1). This study was approved by the Institutional Review Board of the Icahn School of Medicine at Mount Sinai, and all the subjects gave written informed consent to participate.

MRI acquisition
All the participants underwent MRI on the same 3.0 T scanner (Magnetom Skyra, Siemens, Erlangen, Germany) using a 32-channel head coil, with a constant protocol at baseline and FU, including T2-weighted, T1-weighted, and a single shot gradient echo planar imaging sequence for the resting state. Sequence parameters are reported in SI.

Structural MRI analysis
T2 and T1 lesions were segmented, and lesion volumes (T2 LV and T1 LV, respectively) obtained using a semiautomatic technique (Jim 7, Xinapse Systems, Northants, UK). The T1-weighted images of PMS patients underwent a lesion in-painting procedure prior to the brain extraction and anatomical structures' segmentation.[23] The baseline normalized brain volume (BV), gray matter volume (GMV), and white matter volume (WMV), and the FU percentage BV changes were estimated using SIENAX and SIENA, respectively.[24]
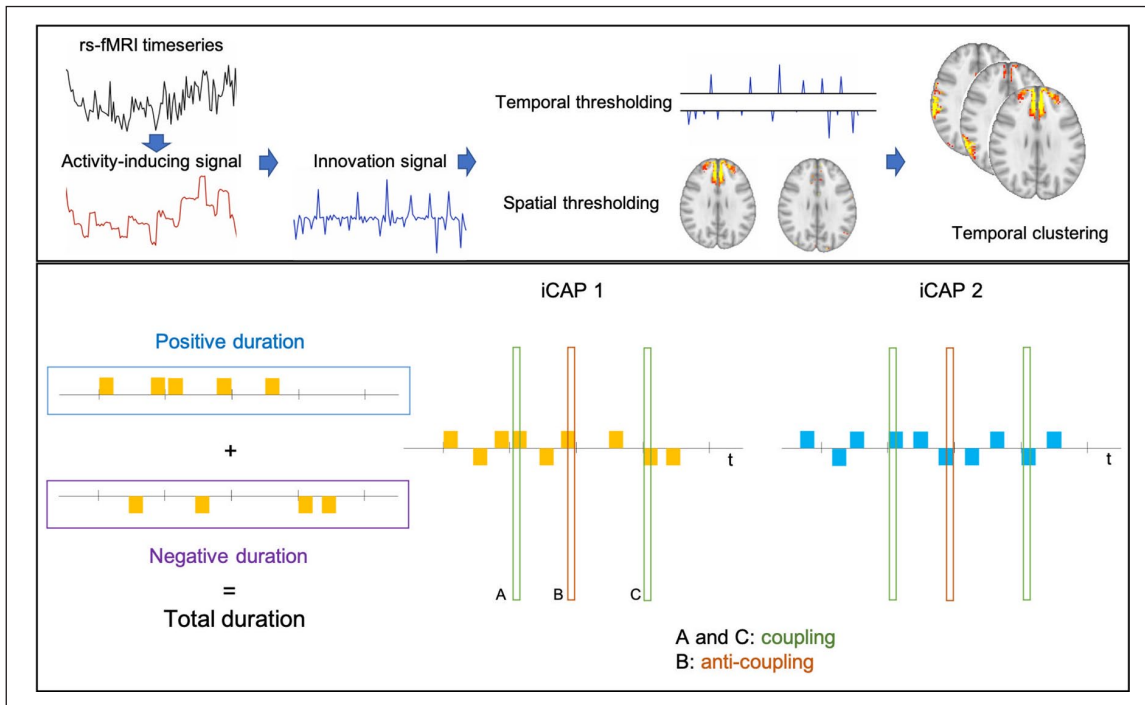
Figure 1. Top: fMRI time-courses undergo a deconvolution step to obtain activity-inducing signals. Innovation signals, obtained by computing the temporal derivative of the activity-inducing signals, enter a two-step thresholding procedure (temporal and spatial) and the resulting frames undergo the temporal clustering step to extract iCAPs. Bottom: the total duration for each iCAP was computed considering both the positive and negative durations, that is, the time-points when that iCAP was active (after thresholding using a cut-off of |1|). The overlap between two iCAPs was measured with the Jaccard score, obtained dividing the number of time-points when the two iCAPs were simultaneously active for the number of time-points when at least one of the two iCAPs was active. The couplings and anti-couplings represented the time-points when the two iCAPs co-(de)activated (A and C) or showed an opposite co(de)-activation (B), respectively.

Functional resting-state MRI preprocessing and innovation-driven-co-activation-pattern analysis

Resting-state functional magnetic resonance imaging (rs-fMRI) data were preprocessed using SPM12 (FIL, UCL, UK), complementary functions of the Data Processing Assistant for Resting-State fMRI (DPARSF),[25] and the Individual Brain Atlases using Statistical Parametric Mapping toolboxes.[26] Preprocessing steps included realignment and discarding of the first 10 volumes, resulting in 390 time-points, co-registration of the T1-weighted and functional images, segmentation of the structural images into white matter (WM), gray matter, and cerebrospinal fluid (CSF). The regression of the nuisance variables, including the average WM and CSF signals and six motion parameters, was performed using DPARSF toolbox. A spatial smoothing (Gaussian kernel of a 6-mm full width at half maximum) was finally applied.

The innovation-driven-co-activation-pattern (iCAP) analysis is a novel technique that allows to derive a set of whole-brain spatial patterns of regions whose activity simultaneously increases or decreases, thus characterized by similar functional dynamic behavior. These are obtained by detecting the *transients* or changes in rs-fMRI activity time-courses and each iCAP represents a brain functional *state* or network. The procedure was performed using a publicly available implementation (https://c4science.ch/source/iCAPs/). For a comprehensive explanation of the methodology, please refer to Karahanoglu et al.,[27] to SI, and Figure 1. The iCAPs retrieved from the baseline sample were fitted into the FU sample, as detailed in SI. From now on, the terms "iCAPs," "networks," or "states" will be used interchangeably to describe the spatial patterns of functional activations derived from the iCAPs' analysis.

iCAPs' temporal properties

For each subject at baseline and FU, we obtained iCAPs' temporal characteristics: (1) the *duration* of overall functional activation, a metric describing the amount of sustained activity (or functional "engagement") for that specific iCAP; for each pair of iCAPs: (2) the *coupling*: same-signed co-activation, and (3) the *anti-coupling*: opposite-signed co-activation (Figure 1). Couplings and

anti-couplings reflect the amount of overlap between the activity, in the same or the opposite signs, respectively, of each iCAPs' pair, and are closely related to the networks' static functional connectivity.

Statistical analysis

Demographic and clinical parameters were compared using unpaired and paired *t*-test between HCs and patients with PMS or between baseline and FU, respectively.

Analysis of covariance (ANCOVA) was used to compare iCAPs' duration between HCs and PMS patients and between CI and CP patients, using age, gender, and education as covariates. A false discovery rate (FDR) correction according to Benjamini et al.[28] was applied. To compare FU and baseline iCAPs' temporal properties, a paired *t*-test within each group and a repeated measure ANCOVA analysis, considering the group × time interaction, were used. Further analyses on coupling/anti-coupling features were computed only for iCAPs whose duration was significantly different in PMS patients compared to HCs. iCAPs' temporal metrics were correlated with T2 and T1 LV using Spearman's rank correlation coefficient.

Partial least squares correlation (PLSC) analysis was used to investigate patterns of correlation between clinical parameters and the temporal properties of iCAPs. Age, gender, and education were stepwise regressed from each variable, except for the normalized cognitive tests *Z*-scores. The toolbox used to run PLSC analysis is publicly available (https://miplab.epfl.ch/index.php/software/PLS). Since only aDMN temporal properties resulted altered in patients with PMS compared to HCs (see section "Results"), we assessed their relationship with clinical disability. Grouped PLSC analyses were performed to assess the different contribution of aDMN metrics to cognitive test scores in CI or CP patients. Moreover, we performed an exploratory analysis to assess any relationship between all iCAPs' durations and clinical test scores.

Data availability

Data supporting the findings of this study will be shared upon request.

Results

Demographic and clinical data

Demographic data for the two groups and clinical data for patients with PMS are reported in Table 1. Table SI 1 reports data for the subjects who underwent FU or dropped out. No significant differences in demographic variables were observed between HCs and PMS, while BV, WMV, and GMV were significantly lower in PMS patients. PMS patients did not differ in terms of any clinical parameter of disability between baseline and FU (Table SI 2). Twenty-four patients were classified as CI at baseline, and the PMS group showed low normalized scores at the BVMT and SDMT tests (see also SI and Figure SI 3).

Spatial properties of iCAPs

Eleven iCAPs with different spatial distribution were obtained and considered for subsequent analysis. The networks represented in each iCAP are shown in Figure 2 and named based on the observed spatial pattern and its overlap with well-known functional networks (see Table SI 3). Specifically, they represented the deep gray matter (DGM), auditory/sensory-motor (Aud/SM), primary visual (PrimVis), executive control network (ECN), anterior default mode network (aDMN), salience network (SAL), temporo-parietal/language (TempPar/Lan), secondary visual (SecVis), precuneus/posterior default mode network (pCun/pDMN), amygdala/temporal pole (Amy/TP), and orbito-frontal cortex (OFC).

Temporal properties of iCAPs: group differences

Baseline. The overall duration of the aDMN was significantly reduced in patients with PMS compared to HCs ($F(1,52) = 10.99$, $p = 0.002$; Figure 3(a) and Table SI 5). A significantly reduced anti-coupling between aDMN and ECN was also observed in patients with PMS compared to HCs ($F(1,52) = 7.2$, $p = 0.010$; Figure 3(b) and Tables SI 6 and 7). No significant differences were detected when iCAPs temporal metrics were compared between CI and CP patients (Tables SI 8–10).

FU. The longitudinal analysis did not reveal differences in iCAPs' durations in PMS patients or HCs at FU versus baseline while the aDMN–ECN coupling significantly increased in PMS patients at FU compared to baseline ($t(18) = -3.86$, $p = 0.001$, Figure SI 4). No significant group × time interaction was found for the 11 network durations or for the couplings/anti-couplings involving the aDMN. A group effect was confirmed at FU for aDMN duration ($F(1,30) = 14.5$, $p = 0.001$) and the anti-coupling between aDMN and ECN ($F(1,30) = 12.0$, $p = 0.002$). Meanwhile, a group effect emerged for Aud/SM duration ($F(1,30) = 13.3$, $p = 0.001$) and the aDMN–Aud/SM anti-coupling ($F(1,30) = 4.2$, $p = 0.049$).

Table 1. Demographic and clinical data at baseline and follow-up.

	Baseline/BS-sub (n = 57/35)			Baseline vs BS-sub	Follow-up (n = 35)		
	HS (n = 25/16)	MS patients (n = 32/19)	p-value[a]	p-value	HS (n = 16)	MS patients (n = 19)	p-value[b]
Age (years)	47.3 ± 8.7/47.9 ± 7.2	50.8 ± 9.9/51.7 ± 9.5	0.17/0.20	0.73	48.9 ± 7.2	52.7 ± 9.4	0.20
Gender (F, M)	10; 15/7; 9	20; 12/13; 6	0.09/0.14	0.67	7; 9	13; 6	0.14
Education (years)	17.2 ± 3.5/17.7 ± 3.9	16.6 ± 2.4/16.8 ± 2.2	0.46/0.43	0.54	17.7 ± 3.9	16.8 ± 2.2	0.43
Disease duration (years)	–	15.4 ± 12.0/14.2 ± 12.3		0.73	–	15.2 ± 12.3	–
EDSS (median; range)	–	5.75 (1.0–6.5)/6 (1.0–6.5)		0.91	–	5; 2.5–6.5	–
SDMT raw score	–	46.3 ± 15.5/48.8 ± 15.7		0.58	–	51.3 ± 13.3	–
SDMT Z-score		–1.41 ± 1.46/–1.14 ± 1.40				–0.74 ± 1.46	–
BVMT-R raw score	–	16.6 ± 8.7/16.5 ± 9.9		0.96	–	19.8 ± 8.9	–
BVMT-R Z-score		–1.90 ± 1.26/–1.83 ± 1.25				–0.84 ± 1.52	–
CVLT-II raw score	–	54.8 ± 13.2/57.3 ± 11.6		0.49	–	58.7 ± 13.3	–
CVLT-II Z-score		0.18 ± 1.35/0.44 ± 1.22				0.73 ± 1.32	–
T25FW	–	8.8 ± 4.4/9.8 ± 5.2		0.46	–	12.1 ± 15.8	–
9HPT	–	32.1 ± 10.4/31.4 ± 11.4		0.80	–	34.2 ± 20.3	–
T2 LV (mL)	–	8.5 ± 10.3/6.8 ± 9.8		0.57	–	7.0 ± 10.0	–
T1 LV (mL)	–	5.9 ± 7.0/4.7 ± 6.9		0.55	–	5.5 ± 8.1	–
GMV (cm³)	779.8 ± 43.6/773.8 ± 49.1	737.3 ± 48.6/730.6 ± 46.5	<0.01/0.01		–	–	–
WMV (cm³)	718.0 ± 37.5/715.1 ± 38.5	658.2 ± 46.6/660.0 ± 51.6	<0.01/<0.01		–	–	–
BV (cm³)	1496.7 ± 699.9/1488.9 ± 732.5	1395.5 ± 826.9/1390.5 ± 822.1	<0.01/<0.01	0.93	–	–	–
PBVC (%)					–0.27 ± 0.41	–0.56 ± 0.72	0.17
Motion (FD_Power)	0.17 ± 0.05/0.16 ± 0.04	0.19 ± 0.08/0.20 ± 0.08	0.18/0.07		0.16 ± 0.04	0.19 ± 0.07	0.14

BS-sub: subsample of 35 subjects at baseline; EDSS: Expanded Disability Status Scale; SDMT: Symbol Digit Modalities Test; BVMT-R: Brief Visuo-spatial Memory Test; CVLT-II: California Verbal Learning Test–Second Edition; T25FW: timed 25-foot walk; 9HPT: 9-hole peg test; T2 LV: lesion volume in T2-weighted sequence; T1 LV: lesion volume in T2-weighted sequence; GMV: gray matter volume; WMV: white matter volume; BV: brain volume; PBVC: percentage of brain volume change; FD: framewise displacement.

All variables are expressed as mean values ± standard deviation if not otherwise specified.

[a]Unpaired *t*-test between HS (n = 25/16) and MS patients (n = 32/19) at baseline.

[b]Unpaired *t*-test between HS (n = 16) and MS patients (n = 19) at FU.
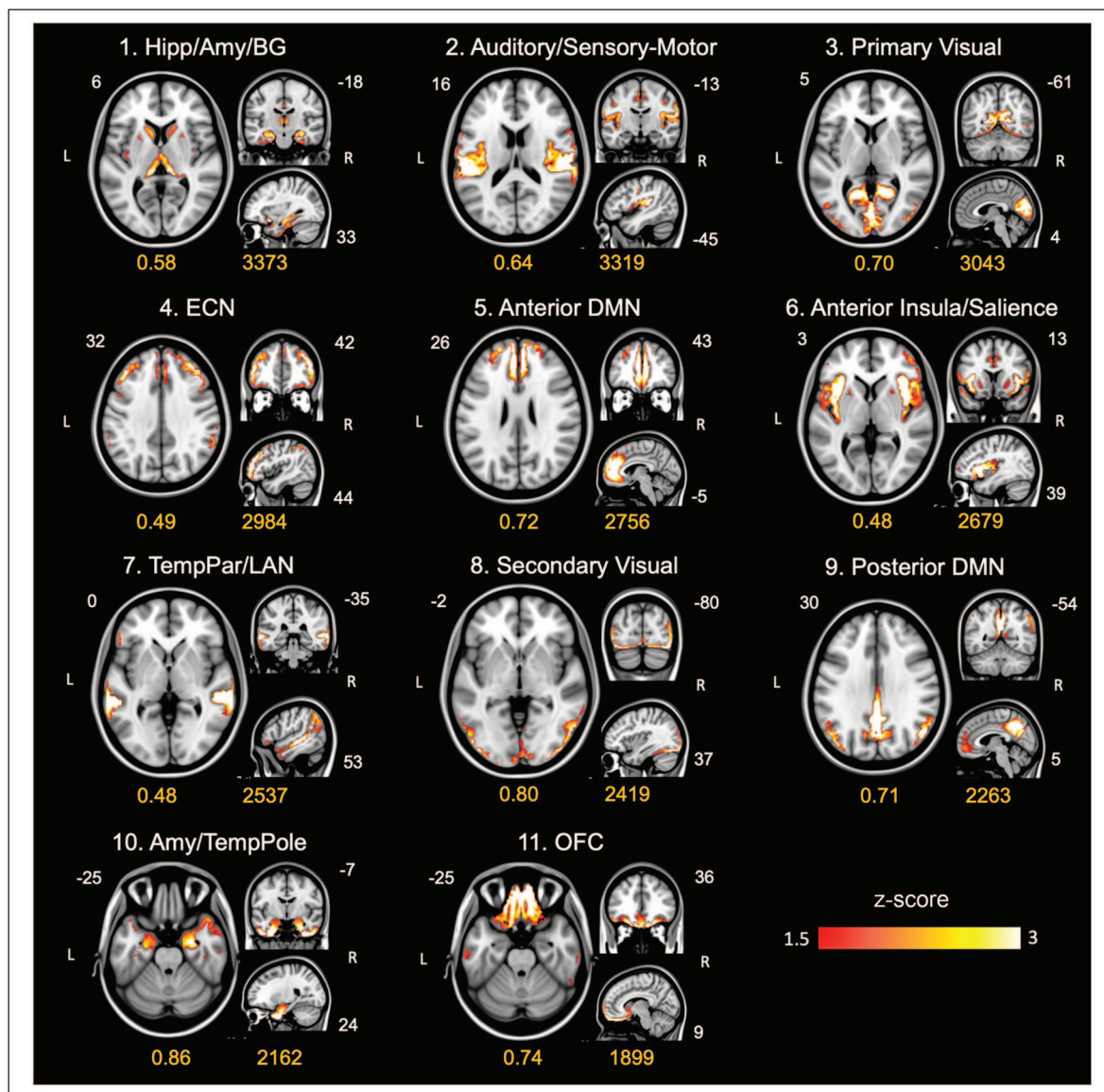
Figure 2. Spatial patterns of the 11 iCAPs retrieved from the analysis on HCs and patients with PMS. Under each iCAP are reported the average consensus (left) and the number of innovation frames assigned to it (right). Coordinates refer to the Montreal Neurological Institute space.

DGM: deep gray matter; Aud/SM: the auditory/sensory-motor; PrimVis: primary visual; ECN: executive control network; aDMN: anterior default mode network; SAL: salience; TempPar/Lan: temporo-parietal/language; SecVis: secondary visual; pCun/pDMN: precuneus/posterior default mode network; Amy/TP: amygdala/temporal pole; OFC: orbito-frontal cortex.

Temporal properties of iCAPs and structural MRI metrics

No significant associations were found between aDMN duration at baseline and T2 or T1 LV. The aDMN–pCun/pDMN anti-coupling and the aDMN-PrimVis coupling positively correlated to T1 LV and T2 LV ($r(27) = 0.40$, $p = 0.04$ and $r(27) = 0.41$, $p = 0.03$, respectively). No correlations were found between aDMN temporal metrics and volumetric parameters.

iCAPs' temporal properties and clinical parameters at baseline

aDMN duration. No significant results were found for EDSS or motor (T25FW and 9HPT) scores. Cognitive scores were associated with aDMN durations ($p = 0.030$; Figure 4). Specifically, a negative correlation was observed for the SDMT and BVMT-R. No significant results emerged from the grouped PLSC analysis (CI and CP patients considered separately).

Figure 3. (a) Bar plots of the duration of the 11 iCAPs in HCs and patients with PMS. (b) Anti-coupling between ECN and aDMN, resulted significantly different between HCs and PMS patients.
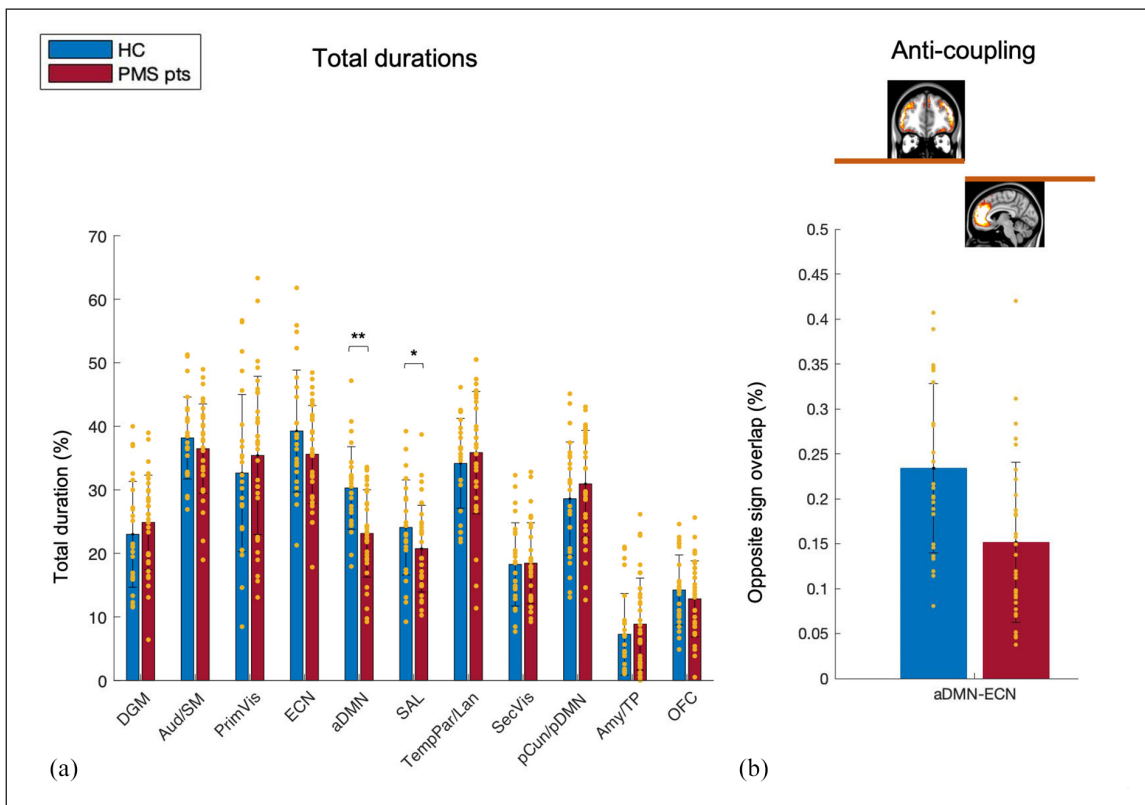DGM: deep gray matter; Aud/SM: the auditory/sensory-motor; PrimVis: primary visual; ECN: executive control network; aDMN: anterior default mode network; SAL: salience; TempPar/Lan: temporo-parietal/language; SecVis: secondary visual; pCun/pDMN: precuneus/posterior default mode network; Amy/TP: amygdala/temporal pole; OFC: orbito-frontal cortex.
*$p < 0.05$; **surviving FDR correction.

aDMN anti-couplings. A significant association between aDMN anti-couplings and cognitive performance was found ($p = 0.005$; Figure 4) in the whole patients group. In particular, SDMT and CVLT-II were related to aDMN-Aud/SM, aDMN-Amy/TP, and aDMN-SAL, with the result mainly driven by positive correlations between aDMN-Aud/SM or aDMN-SAL and CVLT-II score and the negative correlations between aDMN-Aud/SM and SDMT and between aDMN-Amy/TP and CVLT-II. When the grouped PLSC was performed, separating CI and CP patients, we found that aDMN anti-couplings were related to cognitive performance in the CI, but not in the CP group ($p = 0.019$; Figure SI 5).

All iCAPs' durations. This exploratory analysis additionally revealed that the durations of DGM, Aud/SM, TempPar/LAN, and pCun/pDMN were significantly correlated to EDSS and T25FW ($p = 0.027$; Figure SI 6).

Reproducibility analyses
Analyses to verify the reproducibility of the results on study sub-samples are reported in the SI.

Discussion
In this work, we characterized the dynamics of brain functional patterns occurring in patients with PMS, showing that they present changes in the functional activation of the aDMN, and in its interplay with ECN. Such changes were detected also over 1-year FU, in a sample of patients who did not show a significant worsening in clinical disability. Moreover, the dynamic temporal characteristics of the aDMN, namely its reduced sustained activity and the interaction with Aud/SM, SAL, and Amy/TP, explained cognitive disability in patients with PMS.

Regarding the methodology, we chose to apply this specific data-driven approach since it allows to overcome the limitation of the temporal and spatial segregation of
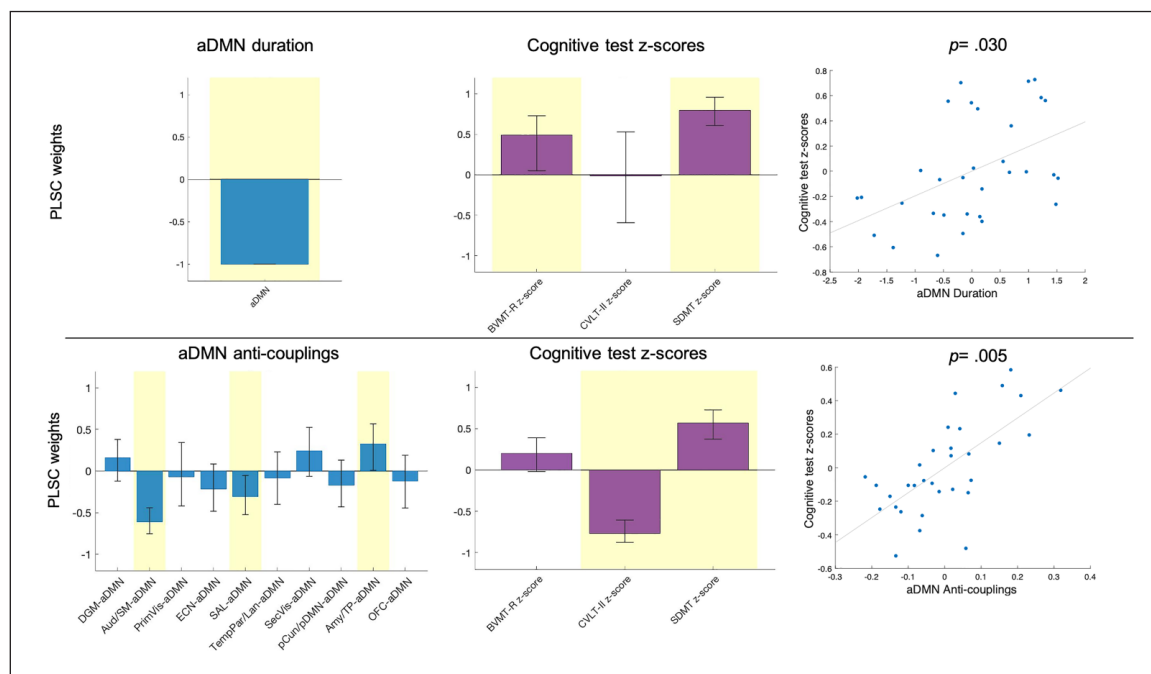
Figure 4. Partial least squares correlation analyses performed to assess the relationship between aDMN duration (top) or anti-couplings (bottom) and cognitive test Z-scores.
DGM: deep gray matter; Aud/SM: the auditory/sensory-motor; PrimVis: primary visual; ECN: executive control network; aDMN: anterior default mode network; SAL: salience; TempPar/Lan: temporo-parietal/language; SecVis: secondary visual; pCun/pDMN: precuneus/posterior default mode network; Amy/TP: amygdala/temporal pole; OFC: orbito-frontal cortex; BVMT-R: Brief Visual Memory Test–Revised; CVLT-II: California Verbal Learning Test–Second Edition; SDMT: Symbol Digit Modalities Test.
In yellow are shown the robust weights after bootstrapping, and the error bars represent bootstrap intervals.

functional networks, characterizing other techniques utilized to investigate functional dynamics, thus better grasping the dynamic interplay between networks over the time of a rs-fMRI sequence. Moreover, these spatial patterns of functional activity have been showed to be reliable across subjects.[29]

As first contribution, this study revealed that the aDMN overall activity duration and its anti-correlation with ECN activity were reduced in patients with PMS. Previous studies showed a reduced aDMN conventional functional activity, related to cognitive disability, in PMS,[3] and highlighted its modifications in other neurodegenerative disorders.[30] The ECN and DMN are usually oppositely engaged and involved in the switching between the "idling" versus "task-oriented" condition. Besides, a disruption in their interaction, mediated by SAL, has been found in several neurodegenerative and psychiatric conditions. Here, we confirm the central role of aDMN dysfunction in the progressive phase of MS and advance the hypothesis that an unbalanced and less dynamic interaction with ECN could be linked to the underlying neurodegenerative process. Intriguingly, the anti-coupling between aDMN and pCun/pDMN as well as its

coupling with the PrimVis network were correlated to lesion load, endorsing the connection between structural damage and functional disruption.

A second relevant finding was the absence of significant changes in functional dynamics over time, accompanied by the lack of clinical worsening. Although we cannot exclude this result could be impacted by attrition bias or learning effect for cognitive tests scores, previous studies in PMS propend for slow changes in clinical and structural imaging metrics[31,32] and the longitudinal variation of brain functional activity might be as gradual. Nonetheless, further longitudinal studies with larger sample sizes are needed to address whether more subtle brain functional modifications occur in the late progressive phase.

As last result, we found a strong relationship between altered functional dynamics of the aDMN and cognitive status. The DMN plays a pivotal role in the cognitive disability in patients with MS[3,6,33] and previous studies on dynamic rs-FC highlighted its role in explaining cognitive impairment, including processing speed.[11,13] Our results indicated that an increased

engagement of the aDMN was associated with a worse cognitive performance. Besides, the cognitive domains expressed by the three tests were differently explained by the interaction between aDMN and the other networks. Namely, a better performance in verbal memory, the cognitive domain less affected in our patients' population, was associated with an increased anti-coupling between aDMN and both Aud/SM and the SAL networks, suggesting that the synchronous opposite-signed activation contributes to ensure a better cognitive functioning, while the opposite was observed for the interaction between aDMN and Amy/TP. Moreover, the aDMN-Aud/SM reduced segregation correlated with a better processing speed, suggesting a compensatory role for the aDMN, likely involving its synchronicity with SM regions.[34] Data from the literature suggest an influence of functional dynamics during rest on the subsequent performance during tasks,[35] hence a possible interpretation of the reduced aDMN dynamics as compensatory, in an attempt to adapt to cognitive tasks demand. Another interpretation entails the aDMN interplay with other networks and the reduced overall duration as an attempt to respond to structural damage reducing the dynamic fluidity to privilege efficiency. When a grouped analysis was performed (SI), the aDMN anti-couplings explained the cognitive status in the CI patients, supporting the idea that changes in behavioral performance are better seized by the dynamic interaction among functional networks. In particular, it emerged an additional relationship between a reduced aDMN–pCun/pDMN anti-coupling and better visuo-spatial memory and processing speed. The latter also correlated to a reduced opposite-signed activity of aDMN with ECN and increased anti-correlation with the higher visual network.

An additional exploratory analysis (SI) showed a relationship between T25FW and EDSS and the Aud/SM, the TempPar, DGM, and pCun/pDMN activity duration. Although none of these networks temporal properties significantly differed, compared to HCs, their relative changes in functional engagement might underlie the modifications characterizing motor disability in PMS.

This study comes with several limitations. First, the small sample size, thus we cannot exclude type II errors. Second, the high rate of drops-out, which could have biased the results and determined the lack of significant changes we observe over 1-year FU. Third, learning effects could have affected the longitudinal results; thus, the absence of a significant worsening in cognitive tests at FU compared to baseline.

Fourth, motor and cognitive test scores were not available for the HCs, which urges caution in the interpretation of the correlation analyses. Fifth, the use of a multi-band acceleration factor of 7 that could have introduced noise in the rs-fMRI data. Sixth, the lack of an external validation, although the analyses on the sub-samples of this study population proved stable and reproducible results. Seventh, the absence of a population of patients at the earlier stages of the disease, which prevents us to conclude that our findings are specific to the progressive stages of MS.

In conclusion, this study characterized functional brain dynamics in patients with PMS, revealing a reduced engagement of the aDMN *state*, although not detrimental for the cognitive status, which was additionally related to the dynamic interplay between aDMN and other functional networks. Future studies are needed to confirm these findings and to address the relation between functional modifications and local or diffuse structural damage through the different disease stages.

Declaration of Conflicting Interests

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Supplemental material

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