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Functional Connectivity Alterations of the Central Autonomic Network in Multiple Sclerosis

Links to Fatigue and Aerobic Training Effects

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Abstract

Background and Objectives

Autonomic dysfunction is common in multiple sclerosis (MS), and autonomic symptoms are independent contributors to MS fatigue. Aerobic training (AT) has been shown to reduce fatigue severity and to promote functional network reorganization in MS. We explored resting-state (RS) functional connectivity (FC) of the central autonomic network (CAN) in MS, its relationship with fatigue severity, and its modifications after AT vs non-AT.

Methods

Seventy-five patients with MS (38 with relapsing-remitting [RR] and 37 with progressive [P]) underwent 3T RS functional MRI at baseline and at the end of AT (24 training sessions, 2–3 times per week for 2–3 months) or non-AT of equivalent duration. Sixty-seven matched healthy controls (HCs) served as a reference for baseline RS FC. At both visits, all patients were assessed using the Modified Fatigue Impact Scale (MFIS). Seed-based RS FC used core CAN modulatory regions: left/right ventromedial prefrontal cortex (vmPFC), mid-cingulate cortex (MCC), amygdala, hypothalamus, and anterior and posterior insula.

Results

Compared with HCs, patients with MS showed increased baseline RS FC of the right amygdala, anterior insula, and vmPFC with superior frontal gyri, precentral gyri and right precuneus. Considering disease phenotype, we found a divergent behavior of baseline RS FC in the interaction analysis ($p < 0.05$, corrected), with patients with RRMS showing predominantly increased RS FC of the amygdala, anterior insula, and MCC with supplementary motor areas (SMAs) and deep gray matter, whereas patients with PMS showed widespread baseline RS FC decrease, particularly involving posterior insula connections with the cerebellum and parietal regions, and MCC connections with frontotemporal areas. In patients with RRMS, increased baseline CAN RS FC inversely correlated with fatigue severity (r = range −0.33/-0.54, p range = 0.046/0.001), while no significant correlations were found in patients with PMS. In patients who underwent AT, decreased hypothalamic network RS FC at follow-up vs baseline was observed ($p < 0.001$, significant time-by-treatment interaction vs non-AT), with more prominent changes in patients with RRMS compared with patients with PMS. No correlations were found between longitudinal RS FC modifications and concomitant fatigue changes.

Discussion

CAN dysregulation is present in MS, with distinct RS FC abnormalities characterizing patients with RRMS and PMS and correlating with fatigue severity. AT modulates CAN RS FC, particularly in patients with RRMS.

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Supplementary Material

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Glossary

ANS = autonomic nervous system; **AT** = aerobic training; **CAN** = central autonomic network; **CASS** = Composite Autonomic Scoring Scale; **CIS** = clinically isolated syndrome; **EDSS** = Expanded Disability Status Scale; **EPI** = echo planar imaging; **FC** = functional connectivity; **FLAIR** = fluid-attenuated inversion recovery; **fMRI** = functional MRI; **FWE** = family-wise error; **HCs** = healthy controls; **IQR** = interquartile range; **LV** = lesion volume; **MCC** = mid-cingulate cortex; **MFIS** = Modified Fatigue Impact Scale; **MNI** = Montreal Neurological Institute; **MS** = multiple sclerosis; **NBV** = normalized brain volume; **PBVC** = percentage brain volume change; **PMS** = progressive MS; **ROI** = regions of interest; **RR** = relapsing-remitting; **RS** = resting-state; **SMAs** = supplementary motor areas; **vmPFC** = ventromedial prefrontal cortex.

Introduction

Autonomic nervous system (ANS) dysfunction is common in multiple sclerosis (MS) and an underappreciated cause of disability.[1] Dysautonomia can appear early in the disease, yet the Expanded Disability Status Scale (EDSS) is insensitive to autonomic involvement, only accounting for bowel and bladder symptoms.[2] Autonomic symptoms can affect the cardiovascular, secretomotor, gastrointestinal, bladder, and pupillomotor domains and are commonly assessed using the Composite Autonomic Symptom Score (COMPASS-31).[3] Increased autonomic dysfunction, as demonstrated by high COMPASS-31 scores, has been shown to correlate with more severe disability, with lowest scores seen in patients with clinically isolated syndrome (CIS) and highest in patients with progressive MS (PMS).[4]

Objective measures of dysautonomia are obtained using a standardized battery of autonomic function tests (tilt test, deep breathing, Valsalva maneuver, sudomotor testing), giving rise to a Composite Autonomic Scoring Scale (CASS) score, which also correlates with the EDSS score.[5] Studies show that laboratory-confirmed sympathetic dysfunction increases in prevalence from relapsing-remitting (RR) to PMS (36%–61%).[6] An increase in adrenergic activity during relapses is thought to lead to adrenergic failure.[7] Parasympathetic dysfunction is found in 5% of patients with CIS and 20% of those with PMS.[8]

Among MS symptoms, fatigue is one of the most common, with a prevalence of up to 83%.[9] Recent studies have found a significant association between fatigue and autonomic symptoms.[10] Objective autonomic function tests correlate with the most peripheral COMPASS-31 domains (secretomotor, gastrointestinal, bladder), but not the central ones (orthostatic intolerance, vasomotor, pupillomotor).[11]

As a consequence, the question of whether the relationship between autonomic dysfunction and fatigue is based on common pathophysiology or it is just a spurious association naturally arises.[12] A subjective-objective mismatch in the evaluation of autonomic dysfunction is common and well described in dysautonomias.[13] In the context of a central disorder like MS, it may be attributed to standard autonomic function testing being based on peripheral autonomic measures, which are inaccurate indicators of central autonomic changes. Functional MRI (fMRI) represents an opportunity to study fatigue and the central control of autonomic function in MS. Many MRI studies of fatigue in MS show that fatigue arises from altered functional recruitment and connectivity of brain networks rather than from structural alterations.[14,15]

A central autonomic network (CAN) was first described by Benarroch,[16] extending the traditional ANS 3-component model (sympathetic, parasympathetic, and enteric) to include higher order brain centers such as the insula, cingulate cortex, ventromedial prefrontal cortex (vmPFC), subcortical regions (e.g., amygdala and hypothalamus), and brainstem nuclei (e.g., nucleus tractus solitarius, dorsal motor nucleus of vagus, and parabrachial nucleus). Aerobic training (AT) has been shown to reduce fatigue severity and to promote functional and structural network reorganization in MS.[17,18] Moreover, AT has been found to positively influence autonomic parameters[19] and to enhance CAN functional connectivity (FC) in the brain.[20]

To better characterize the mechanisms related to ANS dysfunction in MS, we investigated resting-state (RS) FC alterations of the CAN in patients with MS across different stages of the disease and their correlation with the severity of fatigue. The ability of AT (compared with non-AT) in modulating CAN RS FC and concomitant fatigue changes was also explored.

Methods

Patients

Patients were selected from ongoing studies at our Unit to address the study objectives. To be included, patients had to (1) be at least 18 years; (2) have a diagnosis of MS according to 2017 revised McDonald criteria,[21] (3) be relapse and steroid free for at least 3 months before MRI examination; (4) be on a stable MS treatment for at least 6 months; (5) have undergone assessment of fatigue with the Modified Fatigue Impact Scale (MFIS), (6) have no significant medical illnesses or substance abuse interfering with fatigue assessment; (7) not be taking symptomatic treatments for fatigue; and (8) have successfully completed a rehabilitation study targeting

AT (intervention or not, details given further) and undergone both pretraining and post-training clinical and MRI assessments. The final data set included 75 patients with MS, 38 with RRMS and 37 with PMS.

Because patients with RRMS and PMS were studied using different MRI protocols, a total of 67 healthy controls (HCs), divided into 2 subgroups to match by age, sex, and MRI acquisition scanner—patients with RRMS (n = 31: "HC-RRMS") and patients with PMS (n = 36: "HC-PMS") were retrospectively identified from the Neuroimaging Research Unit database, to serve as a reference for baseline RS FC analysis. HCs had no neurologic or systemic diseases affecting the CNS and had a completely normal neurologic examination at the time of MRI.

Clinical Assessment

At the beginning and immediately after the training period, all patients underwent a neurologic assessment with rating of the EDSS[2] score, recording of disease-modifying treatment, and assessment of fatigue using the MFIS,[22] which includes 9 physical items, 10 cognitive items, and 2 psychosocial items.

Intervention

Patients with MS were randomly allocated to 2 groups: an intervention group performing AT (19 with RRMS and 19 with PMS) and a non-AT group (19 with RRMS and 18 with PMS). Both groups performed a total of 24 training sessions. Details of training sessions are reported in eMethods.

MRI Acquisition and Structural MRI Analysis

At baseline and follow-up, all patients were scanned using the same 3.0 T Philips Ingenia CX scanner (Philips Medical Systems, Eindhoven, the Netherlands), using 2 different protocols (using the same protocol in the same patients at both time points). Patients with RRMS and HC-RRMS were scanned using Protocol I while patients with PMS and HC-PMS were scanned using Protocol II. The acquired sequences included the following: (1) RS fMRI, using a T2*-weighted echo planar imaging (EPI) sequence; (2) dual-echo turbo spin echo sequence or 3D fluid-attenuated inversion recovery (FLAIR) sequence for baseline lesion volume (LV) assessment and new T2 lesion count at follow-up; (3) 3D T1-weighted turbo field echo sequence for brain volumetric analysis (eMethods). Brain volumetric analysis included estimation of normalized brain volume (NBV) at baseline and percentage brain volume change (PBVC) at follow-up vs baseline in patients with MS. Details of structural MRI analyses are reported in eMethods.

RS FC Analysis

After RS fMRI preprocessing (details in eMethods), seed regions of interest (ROIs) for core autonomic modulatory regions of the CAN were defined as suggested by previous studies[23] and included the following: mid-cingulate cortex (MCC, Montreal Neurological Institute [MNI] coordinates = 4, 0, 48), left amygdala (MNI coordinates = −22, −8, −16), right amygdala (MNI coordinates = 22, −8, −16), left anterior insula (MNI coordinates = −34, 20, 4), right anterior insula (MNI coordinates = 34, 20, 4), left posterior insula (MNI coordinates = −32, −18, 12), right posterior insula (MNI coordinates = 32, −18, 12), left hypothalamus (MNI coordinates = −5, −18, −8), right hypothalamus (MNI coordinates = 5, −18, −8), and vmPFC (MNI coordinates = −4, 38, −16). A spherical ROI with a radius of 6 mm was centered in the abovementioned coordinates to create seeds. Maps of RS FC were obtained for each patient and time point using a seed-to-voxel correlation approach: the blood oxygenation level–dependent time course was averaged over each seed, and the correlation coefficient between the averaged time course and the time courses of all remaining brain voxels was computed. Correlation coefficients were then converted to z values using the Fisher r-to-z transformation to improve normality, creating a brain RS FC z-score map for each seed, patient, and time point.

Before statistical analysis, RS FC z-score maps were ComBat-harmonized (Empirical Bayes implementation with parametric adjustment) to correct for possible variability involved in data acquisition.[24] Sex, age, and disease status (HC vs MS) were included in the harmonization process to control for interparticipant biological variability. This procedure aimed to improve the homogeneity of the RRMS and PMS cohorts, thereby enhancing the reliability of comparisons between the 2 groups.

Statistical Analysis

Baseline Analysis

T2 LV was log-transformed before analysis. Statistical analysis of demographic, clinical, and structural MRI variables was performed using SPSS software version 29 (Chicago, IL). Between-group comparisons of baseline demographic and clinical variables were performed using the Chi-square, two-sample *t*, or Mann-Whitney test, according to variable distribution. The following between-group comparisons were performed: (1) HCs vs patients with MS, considered as a whole; (2) HC-RRMS vs patients with RRMS; (3) HC-PMS vs patients with PMS; and (4) patients with RRMS vs those with PMS. Baseline T2 LV and NBV were compared across groups (using the abovementioned scheme) using protocol, age, and sex-adjusted linear models. A $p < 0.05$ was considered statistically significant.

Using SPM12 software (Wellcome Center for Human Neuroimaging, London, UK), the following second-level analyses were run on ComBat-harmonized RS FC maps using age-adjusted and sex-adjusted full-factorial models: (1) comparison of RS FC between HCs and patients with MS, considered as a whole; (2) comparison of RS FC among HC, RRMS, and PMS groups. For this latter comparison, each phenotype was first compared with its respective HC group, as previously described, and then, a phenotype-by-group interaction analysis was performed to determine which differences relative to

controls were specific to that phenotype. In the Results section, we focused specifically only on clusters surviving such interaction analysis.

Longitudinal Analysis

Using SPSS 29.0, longitudinal changes of clinical variables were performed using the Wilcoxon signed-rank test, while PBVC was compared between RRMS and PMS cohorts using protocol, age, and sex-adjusted linear models. Regarding RS FC, repeated-measures full-factorial models, adjusted for age and sex, were used to estimate longitudinal RS FC changes. Appropriate contrasts were generated to test (1) within-group RS FC change over time after AT or non-AT interventions, first in the entire MS cohort and then in RRMS and PMS cohorts, and (2) time-by-treatment interaction analysis. In addition, for this analysis, in the Results section, we focused only on clusters surviving such interaction analysis.

Second-level baseline and longitudinal SPM12 analyses were tested at $p < 0.05$ family-wise error (FWE) and cluster-wise corrected for multiple comparisons. For phenotype comparison and longitudinal assessments, given the exploratory nature of the study and the small sample size, clusters significant at $p < 0.001$, uncorrected (cluster extent k = 50 voxels), were also identified.

Correlation Analysis

Average z-scores of abnormal baseline RS FC in RRMS and PMS cohorts were extracted from clusters significant at the phenotype-by-group interaction analysis using REX (www.nitrc.org/projects/rex). Such RS FC z-scores were then entered to SPSS Spearman rank correlation analysis to examine the relationship between phenotype-specific RS FC abnormalities and total and subscales MFIS scores for RRMS and PMS cohorts, separately. Similarly, average z-scores of longitudinal RS FC changes over time in AT and non-AT groups were extracted from clusters surviving at the time-by-treatment interaction analysis and were used to perform correlations between RS FC changes and MFIS score changes after each intervention. A $p < 0.05$ was considered statistically significant.

Standard Protocol Approvals, Registrations, and Patient Consents

The study was approved by the IRCCS Ospedale San Raffaele institutional ethical standards committee on human experimentation and was conducted in accordance with the Declaration of Helsinki. Written informed consent from all the participants was obtained prior to participation in the study.

Data Availability

The corresponding author, who had full access to all study data, assumes responsibility for the integrity of the data and the accuracy of the analysis. The anonymized data set used and analyzed in this study is available on reasonable request from the corresponding author.

Results

Baseline Demographic, Clinical, and Structural MRI Characteristics

Demographic, clinical, and structural MRI variables from study participants at baseline are provided in Table 1.

Age was not significantly different between RRMS and PMS cohorts ($p = 0.12$). EDSS scores and disease duration were significantly higher in patients with PMS compared with patients with RRMS ($p < 0.001$). Most patients were on high-efficacy treatment (51.4%).

MFIS scores were significantly higher in patients with MS compared with HCs ($p < 0.001$) and in patients with PMS compared with patients with RRMS ($p = 0.01$).

T2 LV was significantly higher in RRMS and PMS cohorts compared with their respective HC group but was not significantly different between RRMS and PMS cohorts ($p = 0.06$). NBV was significantly lower in RRMS and PMS cohorts compared with their respective control groups and in patients with PMS compared with patients with RRMS ($p < 0.001$).

Baseline RS FC Abnormalities of the CAN in Patients With MS

Effect of Disease

Group-level spatial maps of CANs derived from the seed regions considered in this analysis are shown in Figure 1. Compared with HCs, patients with MS showed increased RS FC ($p < 0.05$, FWE corrected, Table 2) of the right amygdala with the right frontal cortex, precuneus, and posterior cingulate cortex, as well as increased RS FC of the right anterior insula with bilateral precentral and postcentral gyri and bilateral supplementary motor area (SMA). They also showed increased RS FC between the vmPFC and left superior frontal cortex.

Effect of Clinical Phenotype

Results of CANs RS FC differences among phenotypes are summarized in Table 3. Overall, results indicated distinct and divergent patterns of RS FC abnormalities between RRMS and PMS cohorts (Figure 2).

More specifically, focusing on regions significant at phenotype-by-group interaction, patients with RRMS exhibited predominantly increased RS FC vs HCs, particularly involving the left amygdala, right anterior insula, and MCC, which showed stronger RS FC with the right putamen and bilateral SMA ($p < 0.05$, FWE corrected). At $p < 0.001$ uncorrected, additional clusters of RS FC increase were found between the bilateral hypothalamus and precuneus, between the MCC and frontotemporal regions, between the left anterior insula and right middle frontal gyrus, and between the left posterior insula and left superior occipital gyrus.

Conversely, patients with PMS exhibited a widespread reduction of RS FC compared with HCs: such a decrease was especially pronounced in the connections of the posterior insula with bilateral cerebellum and parietal lobes, as well as in the connections of MCC with frontotemporal cortical areas ($p < 0.05$, FWE corrected). At $p < 0.001$ uncorrected, additional clusters of RS FC decrease were found between the left anterior insula and the left cerebellum and anterior cingulate cortex, between MCC and the right amygdala, between the bilateral posterior insula and frontal regions, and between MCC and the right orbitofrontal cortex. Finally, a circumscribed RS FC increase ($p < 0.001$, uncorrected) was found in patients with PMS vs HCs in the connections between the left amygdala, left anterior insula, and left caudate nucleus.

Follow-Up Clinical and Structural MRI Characteristics

No patient changed treatment during the follow-up, and EDSS scores remained stable. No new T2 lesions were detected in any patient with MS. The mean PBVC was −0.11% in the RRMS cohort and −0.25% in the PMS cohort (RRMS vs PMS; $p < 0.001$).

Nineteen with RRMS and 19 with PMS underwent AT, while the remaining 19 with RRMS and 18 with PMS underwent non-AT. Compared with baseline, patients with RRMS undergoing AT showed a significant reduction in fatigue severity (median total MFIS score: baseline = 31 [interquartile range (IQR) = 26.0; 50.0]; follow-up = 18 [IQR = 14.0; 28.0], $p < 0.001$). In this group, a significant score reduction was also noted in the physical and cognitive MFIS subscales ($p < 0.001$). No significant changes of MFIS total scores and subscores were detected in patients with RRMS performing non-AT, as well as in AT and non-AT PMS groups.

CAN RS FC Changes Over Time in Patients With MS According to the Received Intervention

Results of RS FC changes over time within seed-based reconstructed CANs, according to the received treatment (first considering all patients with MS together and then divided according to clinical phenotype) and surviving the time-by-group interaction analysis, are presented in Table 4 and Figure 3.

When considering all patients with MS who completed AT, compared with non-AT, we found a significant RS FC decrease at follow-up vs baseline between the right hypothalamus and the right MCC ($p < 0.001$, uncorrected). No RS FC changes were found in patients with MS who completed non-AT, compared with AT.

In patients with RRMS undergoing AT, compared with non-AT, a significant decrease in RS FC over time ($p < 0.05$, FWE corrected) was found between the left hypothalamus and right medial superior frontal cortex, as well as between the right hypothalamus and left angular gyrus.

In patients with RRMS undergoing non-AT, compared with AT, a significant decrease in RS FC over time ($p < 0.001$, uncorrected) was found between the left hypothalamus and right insula.

In patients with PMS who underwent both treatments, no RS FC changes were detected at $p < 0.05$ FWE corrected. At $p < 0.001$ uncorrected, a significant decrease in RS FC over time was found in patients with PMS who underwent AT compared with non-AT between the right hypothalamus, right putamen, and right MCC. The opposite contrast showed no difference.

Analysis of Correlations

At baseline, in patients with RRMS, increased RS FC between the left amygdala and right putamen correlated with lower cognitive MFIS scores ($r = -0.35$, $p = 0.03$); increased RS FC between MCC and left SMA correlated with lower total ($r = -0.49$, $p = 0.002$), cognitive ($r = -0.50$, $p = 0.001$), and physical ($r = -0.38$, $p = 0.02$) MFIS scores; and increased RS FC between the right anterior insula and right SMA correlated with lower total ($r = -0.48$, $p = 0.003$), cognitive ($r = -0.33$, $p = 0.046$), and physical ($r = -0.53$, $p < 0.001$) MFIS scores. No significant correlations were found between baseline RS FC abnormalities within reconstructed CANs and fatigue severity in patients with PMS.

No significant correlations were found, in any of the study groups, between RS FC changes over time in patients with MS after training (considered as a whole or divided into phenotypes) and concomitant changes in MFIS scores.

Discussion

In this study, we used RS fMRI to investigate CAN FC abnormalities in patients with MS, the association between FC alterations and fatigue severity, and changes in CAN FC after AT vs non-AT treatment, to determine whether AT modulated RS FC changes related to concomitant fatigue modifications. Our results revealed that CAN dysregulation is present in MS, with distinct and divergent RS FC abnormalities in patients with RRMS and PMS. At baseline, increased CAN RS FC inversely correlated with fatigue severity in patients with RRMS, while no significant correlations were found in patients with PMS. Partial modulation of CAN FC after AT (significant vs non-AT treatment at interaction analysis) was observed in patients with MS, which was more prominent in patients with RRMS, but without significant correlations with concomitant fatigue changes.

To our knowledge, this is among the first investigations demonstrating alterations in CAN RS FC in patients with MS.

Table 1 Baseline Demographic, Clinical, and Structural MRI Features of the Study Groups

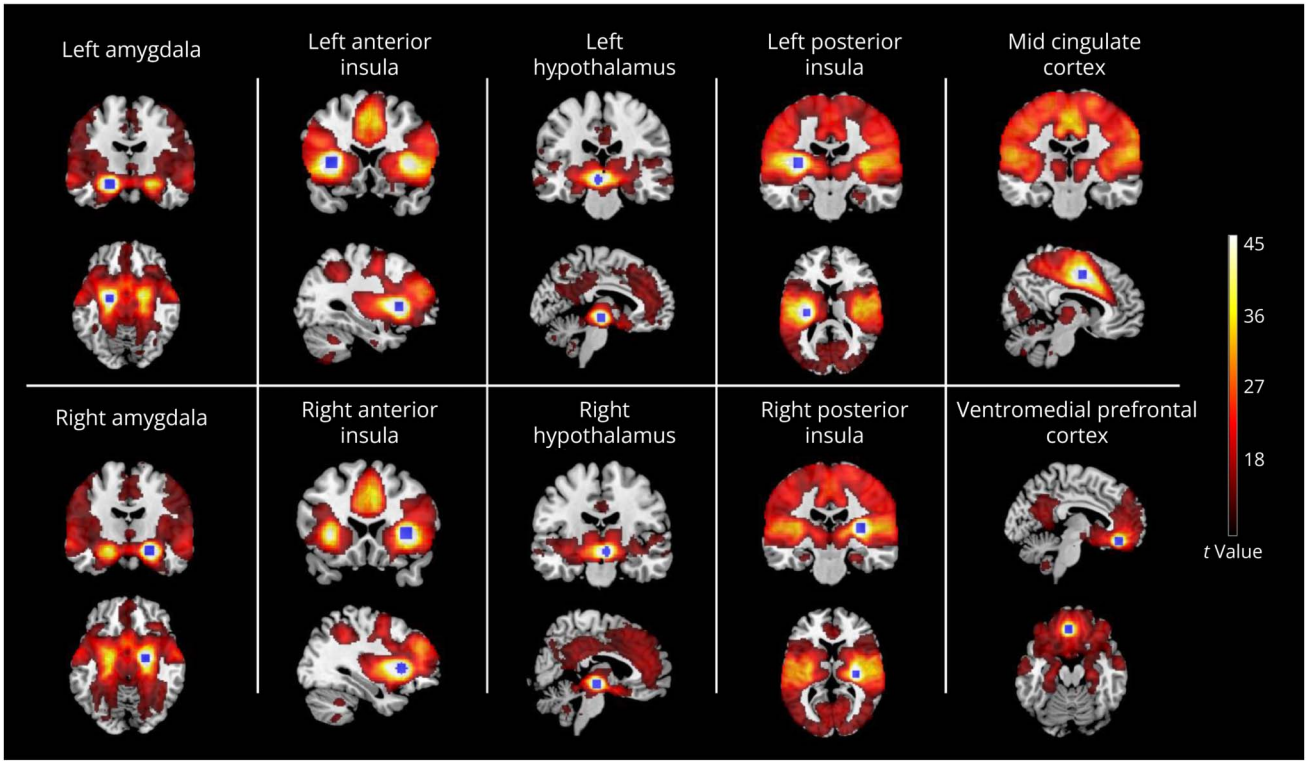
	HC	MS	*p* Value	HC-RRMS	RRMS	*p* Value	HC-PMS	PMS	*p* Value	p value[g]
N	67	75	—	31	38	—	36	37	—	—
Mean age (SD) [y][a]	44.9 (12.2)	49.8 (6.8)	**0.013**	39.5 (11.7)	43.7 (7.3)	0.10	49.5 (10.8)	50.8 (6.1)	0.41	0.12
Sex (M/F) (%)[b]	20/47 (29.9/ 70.1)	22/53 (29.3/ 70.7)	0.97	7/24 (22.6/ 77.4)	10/28 (26.3/ 73.7)	0.72	13/23 (36.1/ 63.9)	12/25 (32.4/ 67.6)	0.74	0.56
Median EDSS score (IQR)[a]	—	4.0 (2.0; 6.0)	—	—	2.5 (1.5; 3.0)	—	—	6.0 (4.5; 6.5)	—	**<0.001**
Median disease duration [y] (IQR)[a]	—	18.4 (9.4; 25.8)	—	—	13.9 (6.7; 22.1)	—	—	21.4 (15.2; 29.1)	—	**0.002**
Median baseline MFIS score (IQR)[a]	14.0 (0.0; 47.0)	43.0 (4.0; 79.0)	**<0.001**	14.0 (6.0; 26.0)	30.5 (4.0; 72.0)	**<0.001**	14.0 (9; 26)	47.0 (16.0; 79.0)	**<0.001**	**0.01**
Median baseline MFIS cognitive score (IQR)[a]	7.0 (0.0; 24.0)	18.0 (0.0; 37.0)	**<0.001**	7.0 (4.0; 13.0)	16.5 (0.0; 31.0)	**0.006**	6.5 (5.0; 14.8)	18.0 (0.0; 37.0)	**<0.001**	0.09
Median baseline MFIS physical score (IQR)[a]	5.0 (0.0; 28.0)	21.0 (2.0; 36.0)	**<0.001**	3.0 (1.0; 11.0)	18.5 (2.0; 36.0)	**<0.001**	6.0 (3.3; 14.8)	24.0 (10.0; 36.0)	**<0.001**	**0.01**
Median baseline MFIS psychosocial score (IQR)[a]	1.0 (0.0; 5.0)	4.0 (0.0; 8.0)	**<0.001**	0.0 (0.0; 2.0)	3.0 (0.0; 8.0)	**<0.001**	1.0 (0.0; 2.0)	4.0 (1.0; 8.0)	**<0.001**	**0.02**
DMT[e] (%): none/MET/HET[b]	—	5 (6.8)/31 (41.9)/38 (51.4)	—	—	0 (0.0)/20 (52.6)/18 (47.4)	—	—	5 (13.5)/11 (29.7)/21 (56.8)	—	**0.02**
Median baseline T2 LV [mL] (IQR)[c,d]	0.0 (0.0; 0.0)	5.7 (2.5; 1.3)	**<0.001**[f]	0.0 (0.0; 0.0)	4.3 (1.3; 9.1)	**<0.001**	0.1 (0.0; 0.5)	8.0 (2.1; 4.5)	**<0.001**	0.06
Mean baseline NBV [mL] (SE)[c]	1,548 (6)	1,473 (6)	**<0.001**[f]	1,555 (9)	1,488 (7)	**<0.001**	1,546 (7)	1,454 (7)	**<0.001**	**0.001**

Abbreviations: DMT = disease-modifying therapy; EDSS = Expanded Disability Status Scale; FDR = false discovery rate; HCs = healthy controls; HET = high-efficacy treatment; IQR = interquartile range; MET = medium-efficacy treatment; LV = lesion volume; MS = multiple sclerosis; NBV = normalized brain volume; NGMV = normalized gray matter volume; NWMV = normalized white matter volume; P = progressive; RR = relapsing-remitting; SE = standard error; WM = white matter.
p values are FDR corrected according to the Benjamini-Hochberg procedure considering the overall number of tests performed. Statistically significant *p* values ($p < 0.05$) are highlighted in bold.
[a] Two-sample *t* test or Mann-Whitney *U* test according to variable distribution.
[b] Chi-square test.
[c] Age and sex-adjusted linear models. Mean values represent estimated marginal mean (SE) derived from the models.
[d] Comparison performed on logarithmic scale.
[e] Age, sex, and protocol-adjusted linear models. Mean values represent estimated marginal mean (SE) derived from the models.
[f] DMT classification: MET = glatiramer acetate, interferon β, teriflunomide, or dimethyl fumarate; HET = S1PR modulators, cladribine, natalizumab, alemtuzumab, and anti-CD20.
[g] RRMS vs PMS.

Standard autonomic testing primarily assesses the peripheral autonomic nervous system, and reliable methods to evaluate central autonomic control are still lacking.[12] The ability of fMRI to noninvasively assess brain connectivity makes it ideally suited to investigate central autonomic control and its pathophysiologic alterations in the context of MS.

When examining the effect of phenotype, patients with RRMS showed increased RS FC within reconstructed CANs, whereas patients with PMS exhibited phenotype-specific widespread decreases, highlighting the importance of analyzing these phenotypes separately in future studies. From studies on the mechanisms of dysautonomia in MS,[7,8] a different pattern of autonomic abnormalities in patients with RRMS and PMS was expected. Indeed, while inflammatory disease activity is associated with sympathetic nervous system dysfunction, parasympathetic nervous system dysfunction becomes more prominent as disease progresses.[25] Although some preliminary fMRI studies using autonomic function tests have attempted to identify which regions of the CAN are associated with sympathetic vs parasympathetic nervous system activity, the findings remain inconclusive. Most brain areas appear to be involved in both systems, resulting in significant overlap.[26] Consequently, it is still not possible to interpret our phenotype-specific results as reflecting sympathetic rather than parasympathetic dysfunction. Of interest, our results show the presence of a certain degree of asymmetry in RS FC of the insular cortex in patients with MS. Older studies with insular electrical stimulation in patients with epilepsy proposed a lateralized control of insular autonomic function, with the right controlling sympathetic and the left controlling parasympathetic output. However, a strict lateralization is debated, due to inconsistent results in more recent fMRI studies.[27] Some studies have proposed a more

Figure 1 CAN Seed Spatial Maps Combined Across Groups



Spatial maps, combined across groups, of central autonomic networks (CANs) produced by seed regions taken into consideration for this analysis (effects of interest derived from SPM12 full-factorial models, $p < 0.05$ family-wise error corrected).

prominent integrative role of exteroceptive and interoceptive inputs in the right compared with the left insula.[28] In line with this, we found a more significant increase in RS FC of the right insula compared with the left in patients with MS and in the RRMS group.

Our results also highlight the previously underreported presence of altered RS FC between the insula and deep gray matter structures and between the insula and MCC with SMA in the MS group. In particular, we found decreased RS FC between the right putamen and the left anterior insula in the RRMS cohort and increased connectivity of the left anterior insula with left caudate in the PMS cohort. The insula develops through a migration pathway from the corticostriatal boundary, which defines the border between the cortex and the ganglionic eminence from which the basal ganglia originate.[29] With the evolutionary emergence of the temporal lobe, the insula acquired a central integrative hub position in the

Table 2 CAN Regions Showing RS FC Differences Between MS and HC Cohorts

Seed region	Contrast	Connected region	MNI coordinates			BA	k extent	*t* value
			x	y	z			
R Amygdala	Patients with MS > HCs	R Medial superior frontal cortex	12	54	30	9	136	4.62
		R Precuneus/R posterior cingulate cortex	2	−62	30	7	182	4.15
R Anterior insula	Patients with MS > HC	L Precentral gyrus/L supplementary motor area	−26	−12	60	6	180	4.95
		R Precentral gyrus/R supplementary motor area	22	2	48	6	133	4.59
		R Postcentral gyrus	24	−36	50	3	138	4.59
Ventromedial PFC	Patients with MS > HC	L Medial superior frontal cortex	0	58	34	9	145	4.54

Abbreviations: BA = Broadmann area; MNI = Montreal Neurological Institute; PFC = prefrontal cortex; L = left; R = right.
Brain regions of central autonomic networks (CANs), reconstructed using seed-based correlation analysis with below-listed regions, showing significant baseline resting-state (RS) functional connectivity (FC) differences between patients with multiple sclerosis (MS) and healthy controls (HCs) (SPM12 full-factorial models adjusted for age and sex). Results are presented at $p < 0.05$, cluster-wise family-wise error (FWE) corrected for multiple comparisons.

Table 3 CAN Regions Showing RS FC Differences Between MS Phenotypes and HCs

Seed region	Contrast	Connected region	MNI coordinates (x, y, z)			BA	k extent	*t* value
			x	y	z			
L Amygdala	RRMS > HC-RRMS	R Putamen	30	0	0	—	156	4.68
	PMS > HC-PMS	L Caudate	−8	10	−14	—	50	3.80
L Anterior insula	RRMS < HC-RRMS	R Putamen	24	14	4	—	80	3.91
	RRMS > HC-RRMS	R Middle frontal gyrus	24	6	46	6	72	4.74
	PMS < HC-PMS	L Cerebellum lobule VII	−26	−74	−50	—	76	4.56
		L Anterior cingulate cortex	0	24	36	24	50	4.08
	PMS > HC-PMS	L Caudate	−18	16	12	—	65	4.40
L Hypothalamus	RRMS > HC-RRMS	R Precuneus	2	−68	22	23	77	4.13
L Posterior insula	RRMS > HC-RRMS	L Superior occipital gyrus	−8	−80	26	19	61	3.56
	PMS < HC-PMS	L Cerebellum lobule VIII	−14	−60	−56	—	120	5.25
		L Cerebellum lobule VI	−10	−76	−14	—	609	5.05
		R Cerebellum lobule VIII	20	−52	−58	—	105	4.94
		B Middle cingulate cortex	−6	−22	78	4	2,488	4.81
		L Postcentral gyrus	−46	−12	44	4	237	4.17
		R Postcentral gyrus	24	−38	66	3	77	3.56
MCC	RRMS > HC-RRMS	L Superior temporal pole	−48	10	−14	38	69	4.78
		L Supplementary motor area	−12	2	64	6	133	4.51
		L Precentral gyrus	−36	−6	64	6	51	4.08
	PMS < HC-PMS	L Insula	−38	−14	12	48	436	5.93
		R Superior temporal gyrus	60	0	−6	21	1822	5.86
		L Precentral gyrus	−48	−6	26	4	693	5.58
		L Cuneus	−10	−78	28	18	493	5.50
		R Amygdala	28	0	−24	36	64	4.94
R Amygdala	RRMS < HC-RRMS	R Hippocampus	24	−28	−10	30	79	4.56
	PMS < HC-PMS	R Rolandic operculum	48	−22	20	48	411	4.43
R Anterior insula	RRMS > HC-RRMS	R Supplementary motor area	22	4	48	6	331	6.11
R Hypothalamus	RRMS > HC-RRMS	L Precuneus	−4	−72	30	7	115	3.98
R Posterior insula	PMS < HC-PMS	L Superior frontal gyrus	−20	−4	68	6	80	5.12
		R Precuneus	0	−2	46	24	766	5.08
		R Cerebellum lobule VIII	24	−52	−58	—	85	5.04
		L Cerebellum lobule IX	−16	−54	−56	—	136	4.77
		L Cerebellum lobule VI	−12	−74	−16	—	344	4.65
		L Precuneus	−12	−42	56	5	100	4.45
		L Insula	−40	−12	2	48	100	4.17
		L Supramarginal gyrus	−56	−26	42	2	285	4.15
		L Postcentral gyrus	−36	−32	50	3	188	3.73
Ventromedial PFC	PMS < HC-PMS	R Orbitofrontal cortex	14	50	−14	11	82	3.71

Abbreviations: BA = Broadmann area; HCs = healthy controls; MCC = mid-cingulate cortex; MNI = Montreal Neurological Institute; PFC = prefrontal cortex; PMS = progressive multiple sclerosis; RRMS = relapsing-remitting multiple sclerosis; L = left; R = right.
Brain regions of central autonomic networks (CANs), reconstructed using seed-based correlation analysis with below-listed regions, showing significant baseline resting-state (RS) functional connectivity (FC) differences in multiple sclerosis (MS) phenotypes compared with their respective healthy control (HC) group (SPM12 full-factorial models, adjusted for age and sex). All reported clusters survive at the phenotype-by-group interaction analysis. An extended version of the table including uncorrected and family-wise error–corrected cluster-wise results is provided in eTable 1.

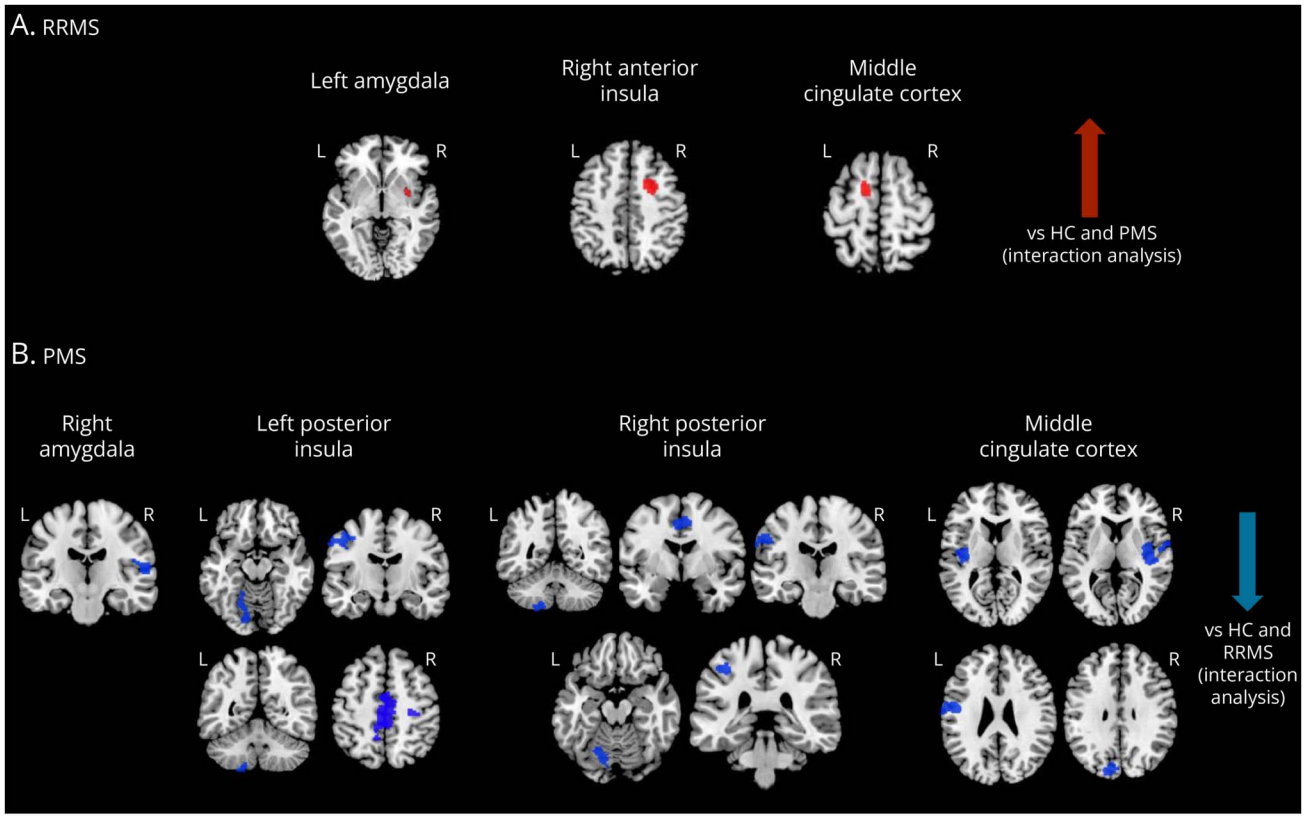
brain. Besides classical cortico-cortical connections, recent studies have also unraveled physiologic structural connections of the insula with deep GM structures.[30] Functional connections of the putamen with the insular cortex point to a previously underreported role of this subcortical structure in autonomic function, which is in line with recent studies.[31] Studies have shown early hypertrophy of the putamen in RRMS and preservation of white matter tracts passing through it, pointing to a compensatory protective role in early MS network pathology.[32,33] Extensive functional connections between the anterior insula and the caudate have also been shown in many studies, in particular on exposure to painful stimuli.[30]

Increased RS FC of MCC and right anterior insula with the SMA was also found in the RRMS cohort in our study. Disruption of SMA activity has recently been found to correlate with perception of physical effort,[34] possibly because of the role of SMA in the creation of a copy of motor command to be sent to the sensory cortex. Indeed, in Parkinson disease, SMA functional alterations have been linked to fatigue as a nonmotor symptom.[35]

In MS, networks undergo continuous changes in topography over the disease course. Earlier studies on connectivity abnormalities in MS show a complex pattern of decreased and increased RS FC.[36] Increases in RS FC within reconstructed CANs found in RRMS might be interpreted as adaptive/compensatory and be lost once the patients develop a progressive phenotype as shown by widespread decrease in RS FC within reconstructed CANs in PMS, possibly leading to network collapse. Notably, we found reduction in RS FC of the cerebellum with the bilateral insula in the PMS cohort. In line with this, a recent study found that cerebellar damage and connectivity changes are more prominent in progressive MS compared with early disease.[37] A cognitive role of cerebellar damage in MS is increasingly recognized but still poorly understood.[38] Moreover, recent studies have pointed out a previously underappreciated role of the cerebellum in autonomic function, showing that reduced connectivity of the cerebellum with other areas of the CAN contributes to altered autonomic cardiovascular control in neurodegenerative diseases,[39] which may also be the case in PMS.

CAN structures have a key physiologic role in interoceptive processing. Interoceptive signals are transmitted from peripheral organs to the brainstem nuclei, from which they are sent to subcortical and cortical CAN structures and ultimately

Figure 2 RS FC Abnormalities in Seed-Based Reconstructed CANs According to Phenotype



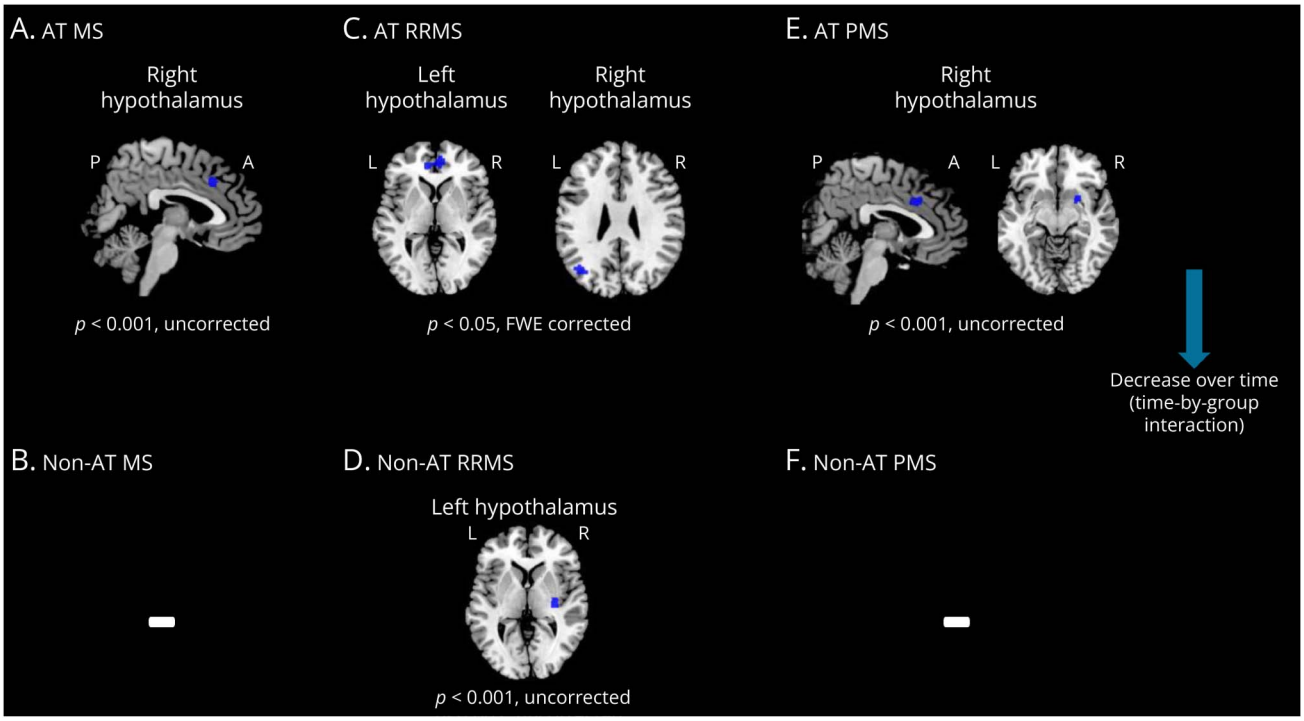
Clusters showing significantly different resting-state (RS) functional connectivity (FC) between patients with relapsing-remitting multiple sclerosis (RRMS) and matched healthy controls (HCs) (panel A), and between patients with progressive multiple sclerosis (PMS) and matched HCs (panel B) in the different central autonomic networks (CANs) reconstructed by seed-region analysis (SPM12 full-factorial models, $p < 0.05$, family-wise error corrected). Only clusters surviving at the at phenotype-by-group interaction analysis are reported. Regions of increased RS FC in patients vs HCs are coded in red, whereas regions of decreased RS FC are coded in blue.

Table 4 CAN Regions Showing RS FC Differences in Patients With MS Over Time Undergoing AT vs Non-AT

Seed region	Contrast	Connected region	MNI coordinates			BA	k extent	t value
			x	y	z			
All AT patients with MS								
R Hypothalamus	Follow-up < baseline	R Middle cingulate cortex	2	24	38	32	54	3.95
AT patients with RRMS								
L Hypothalamus	Follow-up < baseline	R Mid SFG*	4	48	2	10	155	4.56
R Hypothalamus	Follow-up < baseline	L Angular gyrus*	−40	−68	28	39	82	4.13
Non-AT patients with RRMS								
L Hypothalamus	Follow-up < baseline	R Insula	34	−20	6	48	87	4.75
AT patients with PMS								
R Hypothalamus	Follow-up < baseline	R Putamen	24	8	−10	—	62	4.74
		R Middle cingulate cortex	4	16	34	24	50	3.70

Abbreviations: BA = Broadmann area; mid SFG = middle superior frontal gyrus; MNI = Montreal Neurological Institute; PMS = progressive multiple sclerosis; RRMS = relapsing-remitting multiple sclerosis; L = left; R = right.
Brain regions of central autonomic networks (CANs), reconstructed using seed-based correlation analysis with below-listed regions, showing significant changes of resting-state (RS) functional connectivity (FC) over time in patients with multiple sclerosis (MS) undergoing aerobic training (AT) or non-AT. Patients are first considered as a whole and then divided into relapsing-remitting (RR) and progressive (P) phenotypes (SPM12 full-factorial model for repeated measures, adjusted for age and sex). Only clusters surviving at the time-by-treatment interaction analysis are reported.
Results are presented at $p < 0.001$, uncorrected, cluster extent threshold k = 50. Clusters surviving at $p < 0.05$, cluster-wise family-wise error (FWE)–corrected, are marked with *.

Figure 3 RS FC Changes Over Time in Seed-Based Reconstructed CANs According to Clinical Phenotype and Treatment



Clusters showing significant changes of resting-state (RS) functional connectivity (FC) over time in patients with multiple sclerosis (MS) undergoing aerobic training (AT) or non-AT. Patients are first considered as a whole (panels A–B) and then divided into relapsing-remitting (RR, panels C–D) and progressive (P, panels E–F) phenotypes (SPM12 full-factorial models for repeated measures, adjusted for age and sex). Only clusters surviving at the time-by-treatment interaction analysis are reported. Results are presented at $p < 0.001$, uncorrected, cluster extent threshold k = 50, and at $p < 0.05$, cluster-wise family-wise error (FWE) corrected. Regions of decreased RS FC are coded in blue.

to the insula, the primary interoceptive cortex.[40] This allows the brain to model bodily states and anticipate/predict body needs, a process called allostasis.[41] Thus, aberrant CAN connectivity in MS may imply impaired interoceptive processing.

In patients with RRMS, we found several correlations between increased RS FC within CAN areas and lower MFIS scores. MRI studies have shown a prevailing role of the functional rather than the structural damage in the pathogenesis of fatigue in MS.[14] However, how are aberrant network connectivity and development of fatigue linked to each other is not yet known. One answer may reside in the abovementioned interoceptive processing role of the CAN. The CAN has different hierarchical levels. Allostatic predictions are transmitted from lower to higher levels, while prediction errors follow the opposite direction, updating predictions. Altered RS FC of the CAN, through a mismatch between top-down and bottom-up connections, may lead to chronic interoceptive surprise and low allostatic efficiency, underpinning the subjective feeling of fatigue in MS.[42,43]

One peculiar advantage of the ANS is that it can be modulated. Studies show a significant reduction in fatigue severity after AT[18] and a potential of AT in mediating functional network reorganization.[17] A recent study showed that elite athletes had enhanced RS FC within the CAN compared with nonathletes.[20] We aimed to evaluate whether AT, compared with non-AT, was able to modulate the CAN in patients with MS. Fatigue severity decreased after AT vs non-AT in patients with RRMS but not in patients with PMS. After AT vs non-AT, we found a significant RS FC decrease between the right hypothalamus and the right MCC in patients with MS and decreased hypothalamic network RS FC in patients with RRMS. However, such FC change did not correlate with the decrease in fatigue severity. In patients with PMS, we detected no significant RS FC changes after AT.

These results indicate the ability of AT to partially modulate CAN FC, at least in patients with RRMS. The absence of modulation in patients with PMS might be explained by reduced/exhausted neuroplasticity. If alterations in CAN RS FC contribute to the development of fatigue, it could be hypothesized that AT provides insufficient modulation of the CAN, and that a more robust neuromodulatory intervention may be required to achieve meaningful clinical improvement. For example, a longer AT period or enhancement of AT effects via noninvasive neuromodulation strategies (e.g., transauricular vagal nerve stimulation) may be needed to bring out significant RS FC changes and possibly reduce fatigue severity in patients with MS, deserving future investigations.[44,45]

Our study has some limitations. First, the activity of the ANS is highly dynamic and because of the RS design of our study, FC abnormalities of the CAN only evident on certain states, stressors, or activities may not have emerged. Second, owing to difficulties in proper imaging of brainstem regions using EPI-based RS fMRI, FC between CAN brainstem nuclei and higher-order CAN structures has not been evaluated. In a previous study, we demonstrated that AT effects may be influenced by insular lesions.[46] However, the investigation of specific effects of lesions on CAN dysregulation requires a different study design and was not explored here. Moreover, owing to the lack of data on autonomic testing for the included patients, it was not possible to study the relationship between CAN aberrances and laboratory features of dysautonomia in MS. Finally, the short training period may not have been sufficient to detect RS FC changes, especially in patients with PMS.

In conclusion, CAN dysregulation is evident in MS. Patients with RRMS and PMS show distinct and divergent patterns of CAN RS FC abnormalities, which may reflect different compensatory and maladaptive mechanisms. CAN RS FC alterations are associated with the severity of fatigue, possibly indicating changes in interoceptive processing. After AT, a reduction in hypothalamic RS FC compared with non-AT is observed, with more pronounced effects in patients with RRMS compared with patients with PMS, yielding promise for the neuromodulation of the CAN in MS. Notably, FC changes after AT did not correlate with fatigue improvement, which may reflect insufficient modulation or indicate that stronger, longer, or more targeted interventions are required to induce measurable clinical effects. Longitudinal studies and causal connectivity approaches are needed to validate the hypothesis that aberrant CAN-mediated interoceptive processing has a role in the development of MS fatigue.

Author Contributions

G. Guido: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data. P. Valsasina: drafting/revision of the manuscript for content, including medical writing for content; analysis or interpretation of data. T. Morozumi: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; analysis or interpretation of data. P. Preziosa: drafting/revision of the manuscript for content, including medical writing for content; analysis or interpretation of data. F. Romanò: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; analysis or interpretation of data. M.A. Rocca: drafting/revision of the manuscript for content, including medical writing for content; study concept or design; analysis or interpretation of data. M. Filippi: drafting/revision of the manuscript for content, including medical writing for content; study concept or design; analysis or interpretation of data.

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