Cognitive rehabilitation effects on grey matter volume and Go-NoGo activity in progressive multiple sclerosis: results from the CogEx Trial

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ABSTRACT

Background: Research on cognitive rehabilitation (CR) and aerobic exercise (EX) to improve cognition in progressive MS (PMS) remains limited. CogEx Trial investigated effectiveness of CR and EX in PMS: here, we present MRI sub-study volumetric and task-related fMRI findings.

Methods: Participants were randomized to: "CR plus EX", "CR plus sham EX (EX-S)", "EX plus sham CR (CR-S)", and "CR-S plus EX-S" and attended 12-week intervention. All subjects performed physical/cognitive assessments at baseline, week-12 and 6-months post-intervention (month-9). All MRI sub-study participants underwent volumetric MRI and fMRI (Go-NoGo task).

Results: 104 PMS enrolled at 4 sites participated in the CogEx MRI sub-study; 84 (81%) had valid volumetric MRI and valid fMRI. Week-12/month-9 cognitive performances did not differ among interventions; however, 25-62% patients showed Symbol Digit Modalities Test improvements. Normalized cortical grey matter volume (NcGMV) changes at week-12 *vs* baseline were heterogeneous among interventions ($p=0.05$); this was mainly driven by increased NcGMV in "CR plus EX-S" ($p=0.02$). Groups performing CR (i.e., "CR plus EX" and "CR plus EX-S") exhibited increased NcGMV over time, especially in the frontal ($p=0.01$), parietal ($p=0.04$) and temporal ($p=0.04$) lobes, while those performing CR-S exhibited NcGMV decrease ($p=0.008$). In CR groups, increased NcGMV ($r=0.36$, $p=0.01$) at week-12 *vs* baseline correlated with increased California Verbal Learning Test (CVLT)-II scores. "CR plus EX-S" patients exhibited Go-NoGo activity increase ($p<0.05$, corrected) at week-12 *vs* baseline in bilateral insula.

Conclusions: In PMS, CR modulated GM volume and insular activity. Association of GM and CVLT-II changes suggests GM plasticity contributing to cognitive improvements.

KEY MESSAGES

What is already known on this topic:

- Patients with progressive MS often present severe cognitive deficits, affecting their daily-life activities and quality of life. Cognitive rehabilitation and physical exercise can be effective to improve cognition in MS; however, studies in progressive MS are still scanty.
- MRI is an useful paraclinical tool, which has been employed during various rehabilitation protocols to quantify putative measures of plasticity following intervention.

What this study adds:

- During the CogEx study, cognitive rehabilitation and physical exercise were both effective in improving cognition of progressive MS participants, with no differences among interventions.
- Groups performing cognitive rehabilitation showed increased grey matter volumetry (especially in frontal, parietal and temporal lobes) and insular functional MRI activity *vs* those performing sham cognitive rehabilitation.
- Grey matter volume increase over time was correlated with concomitant improvements of cognitive performances.

How this study might affect research, practice or policy

- Involving progressive MS patients in intervention programs requiring an enriched lifestyle is beneficial for their cognition, independently from treatment.
- Grey matter plasticity may be one of the substrates explaining the observed cognitive improvements.

INTRODUCTION

Cognitive dysfunction is present in a large proportion of multiple sclerosis (MS) patients.[1] One of the most affected domains is information processing speed (IPS); however, visuo-spatial abilities, executive functions and working memory are also involved.[2] Progressive (P) MS often present more severe cognitive deficits than relapsing-remitting (RR) patients.[3]

Cognitive rehabilitation (CR) effectively enhances cognition in MS, with various CR protocols showing benefits in the trained domains, especially in RRMS.[4, 5] Preliminary data in other neurological conditions also report cognitive improvements after physical exercise (EX) rehabilitation;[6] however, evidences in MS are less straightforward.[7] MRI is valuable to assess MS-related abnormalities and was often utilized during rehabilitation to quantify putative measures of plasticity post-intervention.[8, 9] Numerous studies detected functional MRI (fMRI) activity and connectivity changes over time following cognitive/motor rehabilitation in MS, generally in brain regions subserving the trained function, suggesting that functional plasticity mechanisms underlie patients' improvements.[10-12] Results related to structural plasticity are more controversial.[10, 11]

Most studies demonstrating the efficacy of CR, EX and combined CR/EX programs on cognitive functions were conducted in RRMS patients,[7, 13] while investigations in PMS are still preliminary and limited by small sample size, cognitive status heterogeneity, lack of MRI monitoring, and no medium-term observations.[14, 15] To overcome such limitations, we recently conducted "Improving Cognition in People With Progressive Multiple Sclerosis Using Aerobic Exercise and Cognitive Rehabilitation" (CogEx, ClinicalTrials.gov ID: NCT03679468),[16] a multi-arm, randomized, blinded and sham-controlled trial testing the effect of different CR and EX combinations on cognitive functions in PMS patients. CogEx was run from 11 international research centers, and enrolled more than 300 PMS patients with impaired IPS. Even though CogEx results failed demonstrating improved efficacy of combined CR and EX on cognitive performances (especially on IPS, the trial primary endpoint) over either interventions alone,[17] IPS improvements could be seen in a large proportion of participants,[17] ultimately suggesting that keeping PMS

patients active across multiple domains may contribute to cognitive amelioration.[17] In a CogEx sub-group at four selected sites, volumetric MRI and fMRI at all study timepoints were also acquired.[16] Our hypothesis was that modifications in grey matter (GM) volumes and fMRI activity occur in PMS patients following rehabilitation, potentially explaining concomitant cognitive changes. To test this, we acquired 3D T1-weighted MRI scans for tracking volumetry of whole-brain and tissue compartments. We also acquired fMRI scans during a sustained attention task (namely, the Go-NoGo task), already employed in MS to map functional substrates of cognitive impairment[18] and to track longitudinal activity changes after rehabilitation.[12] This paper presents findings of the CogEx volumetric MRI and active fMRI sub-study.

MATERIALS AND METHODS

Ethics committee approval and patient consent

Approval was received from Institutional ethical standards committees on human experimentation at participating sites (protocol ID: 32/2018). Written informed consent was obtained from subjects before participation.

Participants

Four centers participated in the CogEx MRI sub-study: a) IRCCS San Raffaele Hospital (Milan, Italy); b) University of Genoa (Genoa, Italy); c) University of Alabama at Birmingham (Birmingham, Alabama, USA) and d) Kessler Foundation (East Hanover, New Jersey, USA).

Patients were enrolled between 14th Dec 2018 and 2nd April 2022. Inclusion and exclusion CogEx criteria are reported elsewhere[16, 17] and in the Online Supplemental Methods. Among key inclusion criteria, there was a confirmed diagnosis of PMS and impaired IPS basing on Symbol Digit Modalities Test (SDMT) evaluation.

Study design and interventions

CogEx methodology has been previously described.[16, 17] Patients were randomized (1:1:1:1) to four treatment arms: "CR plus EX"; "CR plus sham EX (EX-S)"; "EX plus sham CR (CR-S)" and "CR-S plus EX-S". Participants attended 12 weeks of intervention. Clinical, neuropsychological and MRI assessments were conducted at baseline, immediately following intervention ("week-12") and 6 months post-intervention ("month-9"). CR was provided using the RehaCom program.[16, 17] CR-S consisted of Internet training, based on previous studies.[19] EX consisted of aerobic exercise performed on a recumbent arm-leg step ergometer (NuStep T5XR, Ann Arbor, MI, USA).[16, 17] EX-S was focused on balance training and stretching.[16, 17]

Clinical and neuropsychological assessment

At all timepoints (baseline, week-12 and month-9), experienced neurologists blinded to MRI findings performed a neurological examination with EDSS score rating and disease-modifying treatment recording (baseline only), as well as evaluation of walking capacity (6-minute walking test), physical activity and cardio-respiratory fitness.[16, 17]

At the same timepoints, patients underwent a neuropsychological assessment through the Brief International Cognitive Assessment of Multiple Sclerosis (BICAMS),[20] including the SDMT for IPS evaluation, the Brief Visuospatial Memory Test Revised (BVMT-R) for visual memory evaluation and the California Verbal Learning Test-II (CVLT-II) for verbal memory evaluation. Corresponding z-scores were produced by country-specific regressions basing on normative values.[21, 22] At follow-up, subjects were considered SDMT-improved if their score increased by at least 4 points.[23] SDMT-improvements$\geq$8 points were also tested.[23]

MRI acquisition

Using 3.0 Tesla scanners (IRCCS San Raffaele: Philips Ingenia; University of Genoa and University of Alabama: Siemens Prisma; Kessler Foundation: Siemens Skyra) and standardized guidelines for subjects' positioning, the following brain MRI sequences were acquired: a) sagittal

3D fluid-attenuated inversion recovery (FLAIR); and b) sagittal 3D T1-weighted sequence. Whenever possible, an axial T2*-weighted single-shot echo planar imaging sequence during a Go-NoGo fMRI task (total=160 volumes), was also acquired (Online Supplemental Methods).

MRI analysis

Structural MRI analysis. Focal T2-hyperintense white matter (WM) lesions were identified by a fully automated and validated approach using the 3D FLAIR and 3D T1-weighted as input.[24] Output lesion masks were visually checked (and edited, if necessary) by an experienced observer and T2-hyperintense WM lesion volume (LV) was obtained. At follow-up, new T2-hyperintense lesions *vs* previous scans were counted. At all timepoints, normalized GM (NGMV), cortical GM (NcGMV), normalized WM volume (NWMV) and normalized brain volume (NBV, i.e., the sum of NGMV and NWMV) were measured using SIENAx software on lesion-filled 3D T1-weighted sequences.[25] Five cortical masks (frontal, insular-cingulate, occipital, parietal and temporal) were derived using the AAL atlas.[26] Lobar GM volume was calculated by applying these masks to single-subject GM maps, after back-transformation to native space, and was normalized using the SIENAx scaling factor. Segmentation of subcortical GM was performed using the FSL FIRST tool;[25] volume of these structures was calculated and normalized using FSL SIENAx scaling factor. Given their possible relevance, normalized thalamic volume, normalized hippocampal volume and normalized volume of other deep GM nuclei (NDGMV, i.e., the sum of caudate nucleus, pallidum, putamen, amygdala, and nucleus accumbens) entered subsequent analysis. At follow-up, percentage brain volume change (FSL SIENA) was calculated *vs* previous timepoints. Changes of NcGMV, lobar NcGMV, thalamic, hippocampal and NDGMV were calculated as percentage differences *vs* previous scans. Total NGMV and NWMV changes over time were not assessed, because of possible segmentation instability. Mean percentage change of FSL SIENAx scaling factor at follow-up timepoints compared to baseline was=0.45% (SD=0.76%). To ensure longitudinal consistency,

volumetric assessments were excluded from statistical analysis if the FSL SIENAx scaling factor showed excessive variability (>2 SD compared to the mean) across timepoints.

fMRI analysis. After pre-processing (Online Supplemental Methods), changes in blood oxygenation level dependent contrast during the Go-NoGo task were assessed using the general linear model and the theory of Gaussian fields. The first-level design matrix included motion parameters as regressors; average activations over all blocks were derived with appropriate linear contrasts.

Statistical analysis

Statistical analysis was performed using SPSS (IBM, version 26.0) and SAS 9.0. Descriptives of each intervention group were reported as means (and standard deviations [SD]) or median (and interquartile range) for continuous variables, while categorical variables were reported as frequencies. T2 LV was log-transformed.

First, baseline demographic, clinical and neuropsychological variables were compared between patients participating in the CogEx MRI sub-study and patients not participating, to test representativeness of sub-study population, using ANOVA, Chi-square or Mann-Withney U test, as appropriate. Such tests were also used to compare the four treatment arms (in terms of demographic, clinical, neuropsychological and baseline structural MRI variables) for MRI sub-study patients. Only patients having at least baseline and week-12 valid neuropsychological assessments were considered.

A confirmatory analysis of neuropsychological findings of the main trial[17] was performed. Briefly, number of SDMT correct responses and SDMT, CVLT-II and BVMT-R z-scores were compared between interventions at week-12 using ANOVA models adjusted for baseline scores, while Chi-square tests assessed differences in the SDMT-improvements among treatments.

Age-, sex-, and site-adjusted linear mixed models were used to assess and compare among interventions longitudinal changes of volumetric MRI variables (at week-12 *vs* baseline and at

month-9 *vs* week-12). To estimate mean percentage changes, we used as dependent variable in each model the log-transformed volumes at the 3 timepoints. Intervention group, time and their interaction term were included as independent variables. We accounted for within-subject correlation with a compound symmetry correlation-type structure, according to information criteria. Such analyses were repeated: i) by comparing all participants who received CR (i.e., "CR plus EX" and "CR plus EX-S") *vs* those receiving CR-S (i.e., "EX plus CR-S" and "CR-S plus EX-S"), regardless of the EX assigned; ii) by comparing all participants receiving EX (i.e., "CR plus EX" and "EX plus CR-S") *vs* those receiving EX-S (i.e., "CR plus EX-S" and "CR-S plus EX-S"), regardless of the CR assigned; and iii) by comparing SDMT-improved with not improved patients.

fMRI was analyzed using SPM12 software. One-sample t tests ($p<0.05$, family-wise error [FWE] corrected) assessed average Go-NoGo activity at different timepoints. Between-group comparisons of baseline activity and its longitudinal changes were assessed using age-, sex- and site-adjusted full factorial models for repeated measures. The same models produced F-contrasts assessing time-by-group interaction analysis. Results were tested at $p<0.001$, uncorrected, and at $p<0.05$, FWE corrected. Analyses were repeated to test differences: i) between CR *vs* CR-S patients; ii) between EX *vs* EX-S patients; and iii) between SDMT-improved *vs* not improved patients. Average fMRI activity z-score for significant regions were extracted using the REX toolbox (https://www.nitrc.org/projects/rex/) and used for correlation analysis.

Correlations between longitudinal changes of cognitive scores and concomitant changes of structural/functional MRI variables were assessed using Spearman's rank correlation coefficients.

Data availability statement

Anonymised data are available one year after publication, upon reasonable request. Please make the request to the corresponding author, MAR. A CogEx Committee will review the request for approval. A data sharing agreement will be produced before any data are shared. The study protocol and statistical analysis plan were previously published.[16]

RESULTS

Demographic, clinical, and cognitive characteristics

Figure 1 shows study flowchart. 104 PMS patients were initially included (IRCCS San Raffaele Hospital: n=41; University of Genoa: n=40; University of Alabama: n=13; Kessler Foundation: n=10). Of these, 93 patients (89%) completed baseline and week-12 neuropsychological evaluations and 84 (81%) completed baseline and week-12 structural MRI/fMRI. Seventy-nine PMS patients were right-handed and 5 (6%) were left-handed.

Patients participating in the CogEx MRI sub-study were comparable *vs* those not participating for most of clinical and neuropsychological characteristics (Online Supplemental Table 1).

Table 1 shows the main baseline demographic, clinical and neuropsychological variables of MRI sub-study patients, divided according to treatment allocation. No between-group differences were found.

Table 1. Main baseline demographic, clinical and neuropsychological characteristics of multiple sclerosis (MS) patients participating in the CogEx MRI sub-study, divided according to received intervention. Only patients having baseline and week-12 neuropsychological assessments (n=93) are considered.

	"CR plus EX"	**"CR plus EX-S"**	**"EX plus CR-S"**	**"CR-S plus EX-S"**	**p**
N	24	27	20	22	
Mean age [years] (SD)	51 (8.2)	52.5 (5.8)	52.1 (6.0)	51.7 (7.6)	0.89*
Sex (M/F)	11/13	10/17	6/14	9/13	0.74^{+}
Median EDSS score (IQR)	5.0 (4.0-6.5)	6.0 (4.5-6.5)	5.5 (4.0-6.5)	6.0 (4.5-6.5)	0.72^{++}
Mean disease duration [years] (SD)	14.4 (10.1)	15.4 (9.0)	14.7 (11.3)	19.6 (9.9)	0.29*
Type of MS (Primary/Secondary progressive)	7/17	6/21	5/15	3/19	0.64^{+}

6MWT total distance [m] (SD)	231.9 (146.6)	242.4 (102.5)	244.7 (140.8)	271.7 (140.6)	0.77*
VO_2 peak [mL/min/kg] (SD)	14.8 (5.3)	16.1 (6.0)	15.8 (4.5)	15.1 (7.8)	0.86*
Mean WR_{peak} [W] (SD)	74.5 (29.3)	73.1 (27.8)	76.2 (28.0)	74.7 (35.1)	0.98*
Average % in MVPA (SD)	1.7 (1.9)	1.2 (1.7)	2.3 (3.4)	1.3 (1.3)	0.40*
Education [total years of schooling] (SD)	12.5 (3.7)	13.9 (3.3)	14.2 (2.8)	14.1 (3.5)	0.32*
SDMT – mean number of correct responses (SD)	30.7 (7.4)	31.4 (6.5)	31.7 (6.3)	33.8 (8.3)	0.53*
SDMT z-score (SD)	-2.02 (0.5)	-2.05 (0.7)	-2.01 (0.6)	-1.85 (0.5)	0.67*
CVLT-II z-score (SD)	-1.08 (0.9)	-1.09 (1.2)	-1.01 (1.0)	-0.63 (1.1)	0.39*
BVMT-R z-score (SD)	-0.36 (0.8)	-0.67 (1.4)	-0.15 (1.2)	-0.45 (0.9)	0.45*

*ANOVA model;+Chi-square test; ++Kruskall-Wallis test.

Abbreviations: CR=cognitive rehabilitation; CR-S=sham cognitive rehabilitation; EX=physical exercise; EX-S=sham physical exercise; SD=standard deviation; IQR=interquartile range; M=males; F=females; EDSS=Expanded Disability Status scale; 6MWT=6-minute walking test; WR_{peak}=peak work rate; MVPA=moderate-to-vigorous physical activity; SDMT=Symbol Digit Modalities Test; CVLT-II=California Verbal Learning Test-II; BVMT-R=Brief Visuospatial Memory Test Revised.

Cognitive outcomes

Online Supplemental Table 2 shows cognitive scores of the 93 patients completing baseline and week-12 neuropsychological evaluation. Similarly to the main study,[17] no between-group differences in neuropsychological scores were found among interventions at week-12 and month-9, for any treatment subdivision.

The percentage of patients showing SDMT improvements ranged from 43% to 62% at week-12 and from 25% to 41% at month-9, depending on cut-off, with no difference among any group (Online Supplemental Table 2).

Structural MRI findings

Seven 3D T1-weighted MRI were excluded because of insufficient quality and 7 MRI were excluded because of excessive variability in the FSL SIENAx scaling factor.

Table 2 summarizes lesional and atrophy measures divided according to intervention and grouped for treatment type (i.e., groups performing CR *vs* those performing CR-S, and groups performing EX *vs* those performing EX-S). The distribution of centers among treatment groups was homogeneous (p=range 0.74-0.98, Table 2).

Table 2 Main structural MRI characteristics (baseline, week 12 and month 9) of the 88 multiple sclerosis (MS) patients participating to the CogEx MRI sub-study and having at least baseline and week 12 volumetric MRI scans. Patients were first divided according to intervention, and then grouped according to the received type of treatment (i.e., cognitive rehabilitation (CR) or physical exercise (EX)).

	"CR plus EX"	**"CR plus EX-S"**	**"EX plus CR-S"**	**"CR-S plus EX-S"**	**p**	**CR (i.e., "CR plus EX" and "CR plus EX-S")**	**CR-S (i.e., "EX plus CR-S" and "CR-S plus EX-S")**	**p**	**EX (i.e., "CR plus EX" and "EX plus CR-S")**	**EX-S (i.e., "CR plus EX-S" and "CR-S plus EX-S")**	**p**
N	22	23	19	20		45	39		41	43	
Participants from Centers: San Raffaele/Genoa/Alabama/Kessler (N)	9/9/2/2	10/7/3/3	9/7/2/1	8/7/4/1	0.98^{+}	19/16/5/5	17/14/6/2	0.74^{+}	18/16/4/3	18/14/4/7	0.80^{+}
Mean T2 LV, baseline [ml] (SD)	9.0 (8.5)	12.2 (10.3)	15.9 (11.7)	8.2 (8.6)	0.17*	10.6 (9.5)	12.0 (10.8)	0.90*	12.2 (10.6)	10.3 (9.6)	0.52*
Mean NBV, baseline [ml] (SD)	1477 (68)	1449 (60)	1482 (72)	1503 (57)	0.03*	1463 (65)	1493 (65)	0.02*	1479 (69)	1474 (64)	0.57*
PBVC, week-12 *vs* baseline [%] (SD)	-0.01 (0.7)	-0.17 (0.6)	-0.34 (0.7)	-0.13 (0.4)	0.33*	-0.09 (0.6)	-0.22 (0.5)	0.32*	-0.16 (0.7)	-0.15 (0.5)	0.98*
PBVC, month-9 *vs* week-12 [%] (SD)	-0.46 (0.8)	-0.29 (0.6)	-0.41 (0.6)	-0.25 (0.8)	0.65*	-0.39 (0.7)	-0.34 (0.7)	0.56*	-0.44 (0.7)	-0.27 (0.7)	0.25*
Mean NcGMV, baseline [ml], (SD)	614 (41)	600 (41)	612 (46)	626 (30)	0.22*	607 (41)	619 (39)	0.18*	613 (42)	612 (38)	0.92*
Mean % NcGMV change, week-12 *vs* baseline (estimate, 95% CI)	0.41 (-0.46;1.27)	0.94 (0.10;1.78)	-0.47 (-1.38;0.45)	-0.51 (-1.39;0.39)	0.05**	0.69 (0.09;1.29)	-0.49 (-1.12;0.14)	0.008*	-0.01 (-0.65;0.63)	0.27 (-0.35;0.89)	0.54**
Mean % NcGMV change, month-9 *vs* week-12 (estimate, 95% CI)	-0.08 (-1.02;0.87)	-0.28 (-1.26;0.70)	-0.31 (-1.28;0.67)	0.01 (-0.96;0.98)	0.96**	-0.18 (-0.85;0.49)	-0.15 (-0.83;0.53)	0.94**	-0.20 (-0.89;0.49)	-0.17 (-0.87;0.54)	0.94**
Mean NWMV, baseline [ml] (SD)	663 (40)	652 (30)	673 (45)	676 (43)	0.07*	657 (35)	674 (43)	0.02*	667 (42)	663 (38)	0.42*
Mean HippV, baseline [ml], (SD)	9.4 (1.2)	8.6 (1.6)	9.4 (1.5)	9.9 (1.4)	0.03*	8.9 (1.5)	9.6 (1.5)	0.06*	9.4 (1.3)	9.1 (1.6)	0.48*
Mean % HippV change, week-12 *vs*	1.85	0.77	0.03	0.10	0.67**	1.29 (-0.29;2.89)	0.07 (-1.56;1.74)	0.29**	0.98 (-0.64;2.64)	0.45 (-1.13;2.06)	0.64**

baseline, (estimate, 95% CI)	(-0.42;4.18)	(-1.43;3.01)	(-2.31;2.44)	(-2.18;2.45)							
Mean % HippV change, month-9 *vs* week-12, (estimate, 95% CI)	-0.26 (2.67;2.21)	0.03 (-2.50;2.62)	-1.14 (-3.62;1.39)	1.37 (-1.16;3.96)	0.57**	-0.09 (-1.83;1.68)	0.10 (-1.66;1.90)	0.87**	-0.70 (2.42;1.06)	0.72 (-1.07;2.53)	0.26**
Mean ThalV, baseline [ml] (SD)	19.1 (2.8)	18.3 (2.7)	19.1 (2.3)	20.1 (2.7)	0.14*	18.7 (2.8)	19.6 (2.5)	0.10*	19.1 (2.6)	19.2 (2.8)	0.89*
Mean % ThalV change, week-12 *vs* baseline (estimate, 95% CI)	-0.04 (-0.76;0.69)	-0.27 (0.97;0.44)	0.35 (-0.41;1.12)	0.17 (-0.58;0.92)	0.67**	-0.15 (-0.66;0.34)	0.26 (-0.27;0.78)	0.26**	0.14 (-0.38;0.67)	-0.06 (-0.57;0.45)	0.58**
Mean % ThalV change, month-9 *vs* week-12 (estimate, 95% CI)	-0.56 (-1.35;0.22)	-0.25 (-1.07;0.58)	-0.14 (-0.95;0.68)	-0.40 (-1.21;0.41)	0.88**	-0.41 (-0.97;0.14)	-0.27 (-0.83;0.30)	0.72**	-0.36 (-0.92;0.21)	-0.32 (-0.89;0.26)	0.92**
Mean NDGMV, baseline [ml] (SD)	29.3 (3.8)	27.4 (4.5)	29.2 (2.8)	30.5 (3.3)	0.06*	28.4 (4.3)	29.9 (3.1)	0.08*	29.3 (3.3)	28.9 (4.2)	0.58*
Mean % NDGMV change, week-12 *vs* baseline (estimate, 95% CI)	0.80 (-0.33;1.96)	-0.36 (-1.46;0.75)	-0.79 (-1.99;0.43)	0.35 (-0.83;1.55)	0.22**	0.21 (-0.58;1.00)	-0.20 (-1.05;0.65)	0.48**	0.06 (-0.76;0.90)	-0.03 (-0.84;0.78)	0.87**
Mean % NDGMV change, month-9 *vs* week-12 (estimate, 95% CI)	-1.21 (-2.42;0.01)	-0.20 (-1.41;1.02)	0.14 (-1.13;1.42)	-0.40 (-1.68;0.90)	0.47**	-0.70 (-1.56;0.16)	-0.14 (-1.04;0.77)	0.37**	-0.58 (-1.45;0.30)	-0.29 (-1.17;0.60)	0.65**

[+]Chi-square test; *ANOVA adjusted for age, sex and acquisition scanner; **Linear mixed effect model adjusted for age, sex and acquisition scanner.

Abbreviations: CR=cognitive rehabilitation; CR-S=sham cognitive rehabilitation; EX=physical exercise; EX-S=sham physical exercise; SD=standard deviation; CI=confidence interval; LV=lesion volume; NBV=normalized brain volume; PBVC=percentage brain volume change; NGMV=normalized grey matter volume; NcGMV=normalized cortical grey matter volume; NWMV=normalized white matter volume; HippV=normalized hippocampal volume; ThalV=normalized thalamic volume; NDGMV=normalized volume of other deep grey matter nuclei (see text for further description).

Most of baseline lesional and volumetric characteristics were similar among the four interventions, except for NBV and normalized hippocampal volume (both p=0.03).

The median new T2 lesion number at week-12 and month-9 was 0 (interquartile range=0-0) in all groups.

Considering atrophy, no significant heterogeneity was found in volumetric changes over time among treatment groups (p=range 0.22-0.96, Table 2), except for NcGMV at week-12 *vs* baseline (p=0.05). A *post hoc* analysis revealed that such heterogeneity was mainly driven by increased NcGMV over time within "CR plus EX-S" patients (p=0.02).

When assessing groups performing CR *vs* those performing CR-S, a significantly divergent behaviour was found for NcGMV changes at week-12 *vs* baseline (p=0.008, Table 2 and Figure 2), with the CR group showing NcGMV increase and the CR-S group showing NcGMV decrease over time. The analysis of lobar GM atrophy revealed that NcGMV differences between groups were mainly located in the frontal (p=0.01), parietal (p=0.04) and temporal (p=0.04) lobes (Online Supplemental Table 3 and Figure 2). The remaining structural MRI variables did not show any significant difference between CR and CR-S patients, neither at week-12 vs baseline (p=range 0.26-0.48, Table 2), nor at month-9 vs week-12 (p=range 0.37-0.94, Table 2).

Also, no differences were found for EX *vs* EX-s group comparisons (p=range 0.26-0.94; Table 2) and for SDMT-improved *vs* not improved patients (data not shown).

FMRI findings

Behavioral performances during the Go-NoGo fMRI task were comparable across interventions (Online Supplemental Table 4).

Online Supplemental Figure 1 shows the average fMRI activation, which was mainly located ($p<0.05$, FWE corrected) in frontal, parietal, occipital, temporal and insular cortices and did not differ between interventions ($p<0.05$, FWE corrected).

Table 3 and Figure 3 report longitudinal changes of Go-NoGo fMRI activation in the four intervention groups.

Table 3. Changes over time of functional MRI (fMRI) activation during the Go-NoGo task in patients enrolled in the different intervention groups (*post hoc* t tests from SPM12 full factorial model for repeated measures, adjusted for age, sex and acquisition site, $p<0.001$, uncorrected, cluster extent k=10). Results surviving at $p<0.05$, family-wise error corrected for multiple comparisons, are marked with *. Clusters in **bold** were significant at the time-by-group interaction analysis.

Changes over time of task-related fMRI activation						
Group	Contrast	Areas	BA	MNI space coordinates (x y z)	K	T value
"CR plus EX"	Week-12 > Baseline	-		-	-	-
	Baseline > Week-12	-		-	-	-
	Month-9 > Week-12	-		-	-	-
	Week-12 > Month-9	L medial SFG	32	-12 28 34	52	3.73
"EX plus CR-S"	Week-12 > Baseline	-		-	-	-
	Baseline > Week-12	-		-	-	-
	Month-9 > Week-12	-		-	-	-
	Week-12 > Month-9	-		-	-	-
"CR plus EX-S"	Week-12 > Baseline	L Insula* L Postcentral gyrus **R Insula**	13 48 **13**	-36 -28 26* -50 -14 26 **44 -36 20**	195* 63 **33**	4.51 3.56 **3.55**
	Baseline > Week-12	-		-	-	-
	Month-9 > Week-12	-		-	-	-
	Week-12 > Month-9	-		-	-	-
	Week-12 > Baseline	-		-	-	-

"CR-S plus EX-S"	Baseline > Week-12	L SFG	46	-28 52 16	30	3.89
	Month-9 > Week-12	-		-	-	-
	Week-12 > Month-9	-		-	-	-

Abbreviations: CR=cognitive rehabilitation; CR-S=sham cognitive rehabilitation; EX=physical exercise; EX-S=sham physical exercise; L=left; R=right; BA=Brodmann area; MFG=middle frontal gyrus; SFG=superior frontal gyrus.

In "CR plus EX-S", fMRI activity increased at week-12 *vs* baseline in the left insula ($p<0.05$, FWE corrected), left postcentral gyrus ($p<0.001$, uncorrected) and right insula ($p<0.001$, uncorrected), this latter being significant at time-by-group interaction analysis. Within "CR plus EX", fMRI activity decreased ($p<0.001$, uncorrected) in the left superior frontal gyrus (SFG) at month-9 *vs* week-12. Likewise, within "CR-S plus EX-S", fMRI activity decreased ($p<0.001$, uncorrected) in the left SFG at week-12 *vs* baseline. No changes were detected in "EX plus CR-S".

An increased fMRI activity in the bilateral insula at week-12 *vs* baseline (MNI space coordinates, left: -36 -26 28, k=32, right: 40 -36 20, k=24, $p<0.001$ uncorrected) was also found within patients performing CR, being significant for the right insula at the time-by-group interaction analysis *vs* CR-S patients.

A sensitivity analysis performed by repeating all comparisons with the exclusion of 5 left-handed PMS patients confirmed the previous results (data not shown). Finally, no fMRI differences were found between SDMT-improved and not improved patients (data not shown).

Correlation analysis

In groups performing CR, increased CVLT-II scores at week-12 *vs* baseline correlated with increased NcGMV ($r=0.36$, $p=0.01$).

No further correlations were found between structural and task-related fMRI variables vs concomitant changes in cognitive scores.

DISCUSSION

Here, we analysed volumetric MRI and Go-NoGo fMRI data from CogEx MRI sub-study. After intervention, groups performing CR (and, in particular, the "CR plus EX-S" group) had increased cortical GM volume in frontal, parietal and temporal lobes, and increased insular fMRI activity *vs* those performing CR-S. Cortical GM volume changes correlated with concomitant changes of cognitive performances, suggesting that GM plasticity may partially explain observed cognitive improvements.

In line with the main study,[17] combined CR and EX treatment did not show additional cognitive benefits compared to treatments in isolation or sham treatments. Previous MS reports did not give a definite indication about superiority of combined cognitive/motor training *vs* single-modality trainings;[27, 28] However, since cognitive impairment in MS is due to deficits of communications among multi-modal regions, we hypothesized that a multi-domain rehabilitation including both cognitive and aerobic components would be more effective than single CR/EX interventions. Despite this, the CogEx study did not confirm such an hypothesis. Nevertheless, a large proportion of patients[17] presented enhanced SDMT performances at follow-up, suggesting that involving PMS patients in enriched lifestyle interventions results in cognitive improvements.[17]

Moving to MRI, the most intriguing result pertained to cortical GM changes at week-12 *vs* baseline: they were significantly heterogeneous among the four treatment arms, with an indication towards increased cortical GM volume in "CR plus EX-S" patients. A divergent behaviour was also present when comparing all patients undergoing CR, who exhibited increased GM volume, and those undergoing CR-S, who showed the opposite trend. This is notable, since previous MS studies exploring the effects of CR on GM volumetry found no significant changes.[10, 11] On the other hand, action-observation[29] or resistance training[30] modulated cortical GM volume. The notion that cortical

GM volumetry is relevant for cognition is well-established: studies consistently linked smaller neocortical volumes with cognitive deficits in MS,[31 32] with a preferential cortical involvement in PMS.[32] Longitudinal studies indicated greater neocortical volume decrease in cognitively deteriorating than in stable MS patients.[33] Since GM atrophy development characterizes cognitively worsening MS, the opposite trend (i.e., increased or stable cortical GM volume) might be beneficial for cognitive performances. This is further reinforced by our correlation between increased NcGMV at week-12 *vs* baseline and concomitant CLVT-II changes. Interestingly, lobar GM analysis indicated increased cortical GM volume in the frontal, parietal and temporal lobes. This is noteworthy, since frontal, temporal and parietal regions are relevant for several cognitive functions, including those involved by the cognitive training of this study (i.e., divided and sustained attention, vigilance and concentration).[17]

We found no significant volumetric change for hippocampus, thalamus and other deep GM nuclei, probably because of a relatively small sample size or to inherent measurement variability. However, hippocampal and deep GM atrophy might be more important for explaining cognition in RRMS,[34] where these structures might deplete their reserve for adaptive plasticity early on,[35] rather than in PMS patients, where cortical damage is more relevant.[32, 33] Another factor that might explain this result might be related to deep GM long-standing involvement in atrophy processes: it starts to occur at very early MS stages[36] and is therefore very pronounced in PMS. As such, it is likely that deep GM atrophy is a difficult process to be reversed by rehabilitation programs in this phenotype.

Among fMRI findings, the most relevant result was the increase of Go-NoGo fMRI activity in insular regions after training. The insula is a multimodal brain region being a hub of the salience network, having a key role in integrating information from the default-mode and executive control networks.[37] Furthermore, the insula participates in interoception and cognitive control.[38] As such, an abnormal insular activity in MS has been linked with cognitive disturbances.[39] Our finding of increased insular activity during the Go-NoGo task immediately after CR is in line with recent findings in MS patients remaining cognitively stable after 3 years,[40] while reduced insular

connectivity characterized cognitively deteriorating patients.[40] As such, it is conceivable to hypothesize that an improved insular function might be one of the substrates of the cognitive improvements observed in patients undergoing CR. The absence of significant associations between insular activity and concomitant cognitive changes might indicate that, while reflecting changes in brain activation after CR in patients with PMS, the Go-NoGo task might not be sensitive to improvements in more complex cognitive tests. Nevertheless, future studies exploring insular connectivity in this cohort may provide additional insights into changes taking place in the insular network post-rehabilitation.

This study has some limitations. First, sample size of treatment arms was relatively small: enrolling PMS patients with controlled characteristics and willing to participate in an intensive training program was difficult. Also, the COVID-19 emergency somewhat hampered recruitment.[17] While this did not impact our cognitive findings (the same observations were made on a larger cohort[17]), this might explain the lack of correlation between active fMRI and cognitive metrics. Second, we detected a significant correlation between cortical GM volume and concomitant CVLT-II changes over time in CR patients; however, CVLT-II improvements were not different across treatments, somehow limiting interpretability. Third, left-handedness was not an exclusion criterion. However, a few left-handed patients did not excessively contaminate fMRI findings, as shown by the sensitivity analysis reported in the Results section. Finally, global and lobar structural damage was assessed on 3D T1-weighted scans and, even if we used some precautions to improve consistency of volumetry changes over time, we used a method not optimized for longitudinal assessment. Also, volumetric MRI results did not survive correction for multiple comparisons, thus advocating replication of these findings in larger populations.

To conclude, the CogEx MRI sub-study showed no synergistic effect of CR and EX on cognitive performances or structural MRI and fMRI measures of PMS. However, CR modulated cortical GM volumes (especially in frontal, parietal and temporal lobes) and insular fMRI activity. Also, there was some association between increased cortical volume and improved CVLT-II scores

in groups undergoing CR, suggesting that GM still retains a certain degree of plasticity even in this rather advanced PMS population, and that such plasticity might be one of the substrates explaining observed cognitive improvements.

CLINICAL TRIAL REGISTRATION

ClinicalTrials.gov, NCT03679468; registration date: 20 Sep 2018; date of first patient enrolment: 14 Dec 2018.

AUTHORS CONTRIBUTIONS

M.A. Rocca contributed to study concept, analysis and interpretation of data, and to drafting/revising the manuscript. She also acted as study supervisor. P. Valsasina contributed to analysis and interpretation of data, and to drafting/revising the manuscript. F. Romanò contributed to data collection, interpretation of the data and drafting/revising the manuscript. N. Tedone contributed to data collection, interpretation of the data and drafting/revising the manuscript. M.P. Amato contributed to study concept, data collection and drafting/revising the manuscript. G. Brichetto contributed to study concept, data collection and drafting/revising the manuscript. D.V. Boccia contributed to data collection and drafting/revising the manuscript. J. Chataway contributed to study concept, data collection and interpretation, and drafting/revising the manuscript. N.D. Chiaravalloti contributed to study concept, data collection and interpretation, and drafting/revising the manuscript. G. Cutter contributed to study concept, data collection and interpretation, and drafting/revising the manuscript. U. Dalgas contributed to study concept, data collection and interpretation, and drafting/revising the manuscript. J. DeLuca contributed to study concept, data collection and interpretation, and drafting/revising the manuscript. R. Farrell contributed to study concept, data collection and interpretation, and drafting/revising the manuscript. P. Feys contributed to study concept, data collection and interpretation, and drafting/revising the manuscript. J. Freeman contributed to study concept, data collection and interpretation, and drafting/revising the manuscript. M. Inglese contributed to study concept, data collection and interpretation, and drafting/revising the manuscript. C. Meza contributed to data collection and drafting/revising the manuscript. R.W. Motl contributed to study concept, data collection and interpretation, and drafting/revising the manuscript. A. Salter contributed to study concept, data collection and interpretation, and drafting/revising the manuscript. B.M. Sandroff contributed to study concept, data collection and interpretation, and drafting/revising the manuscript. A. Feinstein contributed to study concept, data interpretation, and drafting/revising the manuscript. He also acted as study supervisor. M. Filippi contributed to study concept, data interpretation, and drafting/revising the manuscript.

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COMPETING INTERESTS

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DATA AVAILABILITY

Anonymised data are available one year after publication, upon reasonable request. Please make the request to the corresponding author, MAR. A CogEx Committee will review the request for approval. A data sharing agreement will be produced before any data are shared. The study protocol and statistical analysis plan were previously published.[16]

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FIGURE LEGENDS

Figure 1. Flowchart showing the main steps of the CogEx MRI sub-study. The number of patients with multiple sclerosis (MS) undergoing each step, as well as reasons for exclusion, are reported.

Figure 2. Results from volumetric analysis. Changes at week 12 vs baseline of normalized cortical grey matter volume (NcGMV), frontal NcGMV, parietal NcGMV and temporal NcGMV in patients performing cognitive rehabilitation (CR, N=45) vs those performing sham cognitive rehabilitation (CR-S, N=39) are shown.

Figure 3. Changes over time of functional MRI activations during the Go-NoGo task in the different intervention groups. Clusters showing significant changes over time of functional MRI (fMRI) activation during the Go-NoGo task in the different intervention groups (*post hoc* t tests from SPM12 full factorial model for repeated measures, adjusted for age, sex and acquisition site, $p<0.001$, uncorrected, cluster extent k=10). Increase of activation is reported using a red-yellow scale, while decrease of activation is reported using a blue-lightblue scale. A) Changes occurring in the "CR plus EX-S" group; B) Changes occurring in the "CR plus EX" group; C) Changes occurring in the "CR-S plus EX-S" group; D) Changes occurring in all CR groups (i.e., "CR plus EX" and "CR plus EX-S"). The blue box highlights the cluster surviving at $p<0.05$, family-wise error corrected for multiple comparisons, while the orange box highlights the cluster significant at the time-by-group interaction analysis. Images are in neurological convention.
Abbreviations: CR=cognitive rehabilitation; CR-S=sham cognitive rehabilitation; EX=physical exercise; EX-S=sham physical exercise.