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Enhanced frontoparietal connectivity in multiple sclerosis patients and healthy controls in response to an intensive computerized training focused on working memory

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ABSTRACT

Background: Working memory (WM) deficits are common in multiple sclerosis (MS) patients. Computerized cognitive training may enhance WM capabilities but its efficacy in MS patients has not been sufficiently explored.
Methods: This study examines the effects of n-back training on cognitive performance and functional connectivity (FC) in 29 MS patients and 29 healthy controls (HC). Baseline (S1) performance on 2- and 3-back tasks and FC within the fronto-parietal network were assessed before randomly splitting the sample into four subgroups: trained MS (MSt, n = 15), trained HC (HCt, n = 14), untrained MS (MSu, n = 14), and untrained HC (HCu, n = 15). The trained subgroups underwent adaptive n-back training (60 min/day; 4 days) and n-back task performance and FC were reassessed in a second session (S2).
Results: As revealed by mixed two-way ANOVAs, trained participants (MSt and HCt) exhibited a significant increase in the number of correct responses and significantly reduced reaction times in S2. These performance improvements were accompanied by an increase in FC in the fronto-parietal pathways and statistically significant correlations between both effects were found.
Conclusions: Computerised WM training results in behavioural and neuroplasticity positive effects that may be useful when trying to prevent or attenuate cognitive decline in MS patients.

1. Introduction

There is growing interest in identifying clinical interventions that could delay or reduce the cognitive deficits in multiple sclerosis (MS) patients. In this regard, a recent meta-analysis has pointed out that computer-based cognitive training programs appear to improve cognitive performance in MS patients (Lampit et al., 2019). As the same meta-analysis has concluded (Lampit et al., 2019), more and well-validated studies are needed to confirm the usefulness of this kind of interventions in preventing or mitigating the cognitive decline observed in MS patients.

In principle, the efficiency of any training/ rehabilitation program can be measured either by observing an improvement in the trained cognitive skills or by exploring the brain's response (neuroplasticity) to rehabilitation programs. However, the effectiveness of cognitive rehabilitation programs only becomes definitively supported when these two kinds of data are linked, producing a real gain in the knowledge about the effects of MS on cognitive competence (Mitolo et al., 2015). On this subject, the neuroplasticity processes induced by cognitive rehabilitation in MS patients have been studied in terms of activation or functional connectivity (FC) changes. Regarding the former, several (but not all) studies have described brain activation increases after cognitive rehabilitation, which in some (but not all) cases were directly correlated with observed improvements in cognitive performance (Chiaravalloti et al., 2012; Ernst et al., 2012; Sastre-Garriga et al., 2011). In the latter case, FC studies present more homogenous results, suggesting that cognitive rehabilitation enhances FC in MS patients (Bonavita et al., 2015; De Giglio et al., 2016; Leavitt et al., 2014; Parisi et al., 2014b, 2014a).

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Although the results of these studies are promising, as a recent review (Mitolo et al., 2015) indicate, they are also inconclusive, perhaps due to the heterogeneity of the selected participants, the diversity and lack of specificity of the rehabilitation approaches used, and other methodological weaknesses (e.g., the selection of outcome measures). Therefore, more studies are needed in this area. Moreover, according to the most recent recommendations (Lampit et al., 2019; Mitolo et al., 2015), this research should assess interventions that are implemented through computerised programs, focus on repeated and controlled practice in very structured sessions, and target just one or a few specific cognitive functions. Regarding the latter, behavioural interventions should be designed to boost basic cognitive processes and functions (e.g. working memory, WM) that subserve higher cognitive abilities and may indirectly promote a generalized enhancement of cognitive performance (Mitolo et al., 2015).

In this respect, previous studies using n-back training task (Covey et al., 2018; Hancock et al., 2015) have described the benefits of specifically implementing this task, not only for WM but also for information processing speed (IPS). We recently conducted an activation-based study to assess the effects of an intensive and adaptive computerised n-back training on the WM capabilities of MS patients (Aguirre et al., 2019). We focused our study on WM because: 1) about 27-44% of MS patients show WM impairments and these impairments affect other cognitive domains, such as IPS, attention, learning capacity and executive functions (Chiaravalloti and Deluca, 2008); 2), only a few studies had previously examined changes in cognitive performance and brain activity following WM training in this clinical population (Covey et al., 2018). Our results revealed that, compared to untrained participants, trained MS and healthy controls (HC) exhibited enhanced 2-back and 3-back task performance, an effect that was correlated with activation changes. Furthermore, we observed that the training-induced improvements in WM were accompanied by improvements in IPS. These findings suggest that MS patients are able to benefit from computerized WM training programs capable of promoting neuroplastic changes within the WM network and that such training program results in enhanced WM and IPS capabilities, two cognitive domains characteristically affected in MS patients (Covey et al., 2018; DeLuca et al., 2004; Forn et al., 2008; Hancock et al., 2015).

In the present study, we sought to confirm and extend these results by investigating whether the effects of this particular WM training program on improving n-back performance is also supported by changes in FC. We focus our intervention in MS patients with no cognitive impairment to evaluate if once MS has been already diagnosed but there are no overt signs of cognitive decline, MS patients still retain the same capabilities and potentialities than HC and equally benefit of cognitive training and repeated practice.

We hypothesized that both trained groups (MS and HC) would present similar increases in FC within some nodes of the WM network, and that these changes would be correlated with an increase in the number of correct responses (CRs) and a reduction in the reaction times (RTs) on the 2- and 3-back tasks.

2. Materials and methods

2.1. Participants

Right-handed patients with no cognitive impairments and diagnosed with definitive relapsing-remitting (RR) MS, according to McDonald criteria, were selected for the study and neurologically assessed using the Expanded Disability Status Scale (EDSS). In order to be included in the study, MS patients had to be free from steroids' treatment and have not experienced any relapse episode in the last 2 months. Patients should not present any other concomitant Central Nervous System pathology or major visual or eye-hand coordination limitations. Moreover, right-handed participants with no neurological or psychiatric dysfunctions made up the control group (HC). Participants (HC and MS) were randomly allocated in different subgroups: MS untrained group (MSu, n = 14), HC untrained group (HCu, n = 15), MS trained group (MSt, n = 15), and HC trained group (HCt, n = 14). All participants gave informed written consent prior to participation and received remuneration for completing the study. The Ethical Committee of *Universitat Jaume I* approved the research project and was conducted in accordance with the Declaration of Helsinki.

As we describe in a previous study (Aguirre et al., 2019), participants were neuropsychologically assessed between 5/7 days prior to the scanner with the following measures: 1) Brief Repeatable Battery of Neuropsychological Tests (BRB-N) validated for the Spanish population; Matrix Reasoning Subtest of the Wechsler Adult Intelligence Scale (WAIS III) to assess the intelligence quotient (IQ); Fatigue Severity Scale (FSS); and Beck Depression Inventory (BDI). Moreover, assessment also included two functional Magnetic Resonance Imaging (fMRI) sessions: baseline session (S1) and post-training session (S2; 7 days later).

2.2. MRI acquisition

Neuroimaging data were acquired on a 1.5T scanner (Siemens Symphony, Erlangen, Germany) in S1 and S2 in this order: 1) Anatomical 3D MPRAGE volumes were acquired, using a T1-weighted gradient echo pulse sequence (TR = 2200ms; TE = 3ms; flip angle = 15°; matrix = 256 × 256 × 160; voxel = 1 × 1 × 1 mm), and for MS patients, a FLAIR sequence (TR = 6000 ms; TE = 354 ms; flip angle = 180°; matrix = 196 × 256 × 160; voxel = 1.05 × 1.05 × 1 mm); 2) During n-back fMRI were acquired with a gradient-echo T2*-weighted echo-planar MR sequence covering the entire brain (TR = 2500 ms; TE = 49 ms; matrix = 64 × 64 × 28; flip angle = 90°; voxel = 3.5 mm^3; slice gap = 4.41 mm). A total of 260 volumes were recorded (Aguirre et al., 2019).

The n-back adapted for fMRI studies is described in Aguirre *et al.* (2019). Inside the scanner, visual stimuli were presented electronically using E-Prime software (Psychology Software Tools, Pittsburgh, PA), professional version 2.0, installed in a Hewlett-Packard portable workstation (screen-resolution 800 × 600, refresh rate of 60 Hz). Participants watched the laptop screen through MRI-compatible goggles (VisuaStim, Resonance Technology, Inc., Northridge, CA, USA). During the task, participants had to give "yes" or "no" motor responses that were collected via MRI-compatible response-grips (NordicNeuroLab, Bergen, Norway). The E-Prime's logfile saved the CRs and RTs for each stimulus for each participant.

2.3. N-back training protocol

Two days after S1, the trained groups came to the university to complete four WM training sessions on consecutive days. Each training sessions had a total duration of 60 minutes and they were distributed in two phases. During the first phase, participants performed WM training, adapted from Jaeggi *et al.* (2008), for 50 minutes. In this phase, participants performed three runs, each composed of eight blocks that varied in WM load (1-back, 2-back, and 3-back). For motivational reasons, the training always started at the low level (1-back load), and the level of n-back of the subsequent block was based on the participant's performance on the previous block. Thus, if the participant had at least 90% CRs, the WM load increased one level (e.g. 90% performance on 2-back tasks increased to 3-back). If the CRs during the block were below 80%, in the subsequent block the WM load decreased one level (e.g. from 2-back to 1-back). In all other cases, the n-level remained constant. Participants were instructed to give manual responses only with their right hand, responding to targets with their thumb and to non-targets with their forefinger. Feedback was introduced after each response for a few seconds, as a coloured circle at the corner of the screen: green meant a correct answer, a red circle represented an error, and blue indicated missing responses. Moreover, at the end of each block, subjects also received additional information about their percentage of correct responses (CRs) and the average reaction time (RT) of their responses.

Finally, participants completed a test phase that consisted of eight blocks of the 2- and 3-back tasks. Subjects received no feedback during this time. Their results on this test were used to evaluate their progress on n-back execution. For more information, see also Aguirre *et al.* (2019).

2.4. Neuroimaging analysis

To define regions of interest (ROIs), we extracted the Talairach coordinates of the specific brain regions related to n-back performance reported in Wang *et al.* (2019) (see also Fig. 1 and Supplementary Table 1), and we converted them to the MNI space using the Mango v4.1 toolbox. After that, we used AAL (Tzourio-Mazoyer et al., 2002) to label the corresponding MNI coordinates. Finally, we localized peak coordinates of each AAL region related to the n-back task in our data, and we defined ROIs as 5 mm spheres using the WFU-PickAtlas toolbox (Maldjian et al., 2003). Data preprocessing and first-level analysis were performed using the CONN-Toolbox v18.4 (Whitfield-Gabrieli and Nieto-Castanon, 2012) for SPM12, implemented in Matlab 2018b. Anatomical and functional images were preprocessed using the default pipeline implemented in the CONN-Toolbox, including realignment, co-registration, spatial normalization to MNI templates, smoothing of 4 mm FWHM, and temporal filtering (0.01Hz-0.08Hz). In order to study FC during the 2- and 3-back tasks, we performed a ROI-to-ROI analysis using the implemented generalized Psychophysiological Interaction procedure (gPPI). A separate multiple regression model was computed for each target voxel BOLD timeseries. Each model included three independent variables: 1) the main psychological factor, in this study it corresponds to the three task conditions effects (0-back, 2-back and 3-back) convolved with a canonical hemodynamic response function; 2) the main physiological factor, which correspond with each seed ROI BOLD timeseries; and 3) the interaction term specified as the product of the previous two factors. Finally, we extracted the first-level ROI-to-ROI connectivity matrices of each n-back condition (0-back, 2-back and 3-back) and contrast matrices were calculated to test the effects of the task conditions (2-back and 3-back) compared to control condition (0-back).

2.5. Statistical analyses

Second level statistical analyses were conducted with the *rstatix* package 20 (Kassambara, 2020) for RStudio (version 1.2.5, RStudio, Inc). More specifically, two-way (Group: HC vs. MS; Training: Untrained vs. Trained) ANOVAs were used to compare the groups of participants on the demographic and clinical variables displayed in Table 1. Female/male proportions in these groups were compared by means of the chi-squared test. N-back performance (CRs and RT) and FC scores were analysed using mixed two-way ANOVAs (group x training x session). Results were considered statistically significant when FWER-corrected *p*-values were below the 0.05 threshold. In these cases, appropriate univariate effect size indexes (η^2 or Cohen's *d*) were calculated. The size of the multivariate between-group differences in the degree of change in FC scores (Δ-FC scores = FC_{S2}- FC_{S1}) during 2- or 3-back performance was also estimated. More specifically, the unbiased Mahalanobis' D, the overlap coefficient, and the probability of superiority (PS) were calculated with the *maha* function (Del Giudice et al., 2012), whereas the

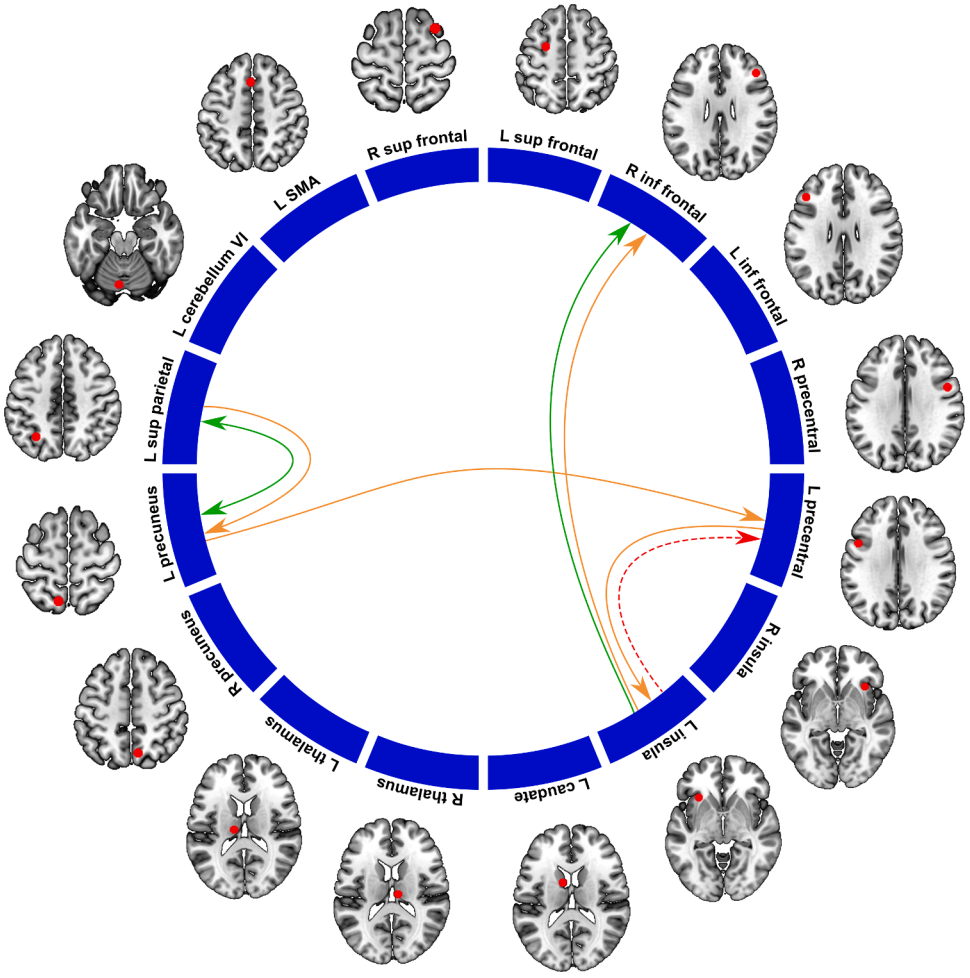

Fig. 1. Connectivity between ROIs during 2- and 3-back tasks. Solid line arrows represent stronger functional connectivity (FC) in trained participants (HCt and MSt) compared to untrained participants (HCu and MSu; Training*Time effects) during the execution of 2-back (orange) and 3-back (green) tasks. The red dashed line arrow represents differences in FC between MSu and MSt (Group*-Training*Time effect) during the 2-back task. Results were $p<0.05$, FWE-corrected. R: right, L: left; SMA: supplementary motor area. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Demographic, clinical and neuropsychological data of all participants

	HC_U (n = 15)	**HC_T (n = 14)**	**MS_U (n = 14)**	**MS_T (n = 15)** (mean ± SD)	(mean ± SD)	(mean ± SD)	(mean ± SD)	**Differences** *T = Training factor G = Group factor TxG = Interaction*
Age[A,B]	34.13 ± 6.07	31.21 ± 8.72	36.14 ± 5.97	35.80 ± 7.3 (25-45)	(24-50)	(22-46)	(22-46)	*T*: $F_{1,54} = 0.76$, p = 0.39 *G*: $F_{1,54} = 3.12$, p = 0.08 *TxG*: $F_{1,54} = 0.48$, p = 0.49
Gender (men/women)	9/6		6/8		3/11		7/8	$\chi^2_{(3)} = 4.51$, p = 0.21
Educational level (1-6 levels)	3.73 ± 1.28	4.71 ± 0.83	3.71 ± 1.49	3.73 ± 1.71				*T*: $F_{1,54} = 1.93$, p = 0.17 *G*: $F_{1,54} = 1.93$, p = 0.17 *TxG*: $F_{1,54} = 1.78$, p = 0.19
EDSS		-		1.80 ± 1.70	1.67 ± 1.51			$t_{27} = 0.19$, p = 0.85
Disease duration (years)	-		-		7.54 ± 5.12	8.33 ± 5.96		$t_{27} = 0.38$, p = 0.85
Total lesion volume (mL)		-		4.39 ± 4.88	2.36 ± 3.56	$t_{27} = 1.28$, p = 0.21		
BPF	0.86 ± 0.01[C, D]	0.85 ± 0.02[C, D]	0.84 ± 0.02	0.84 ± 0.01				*T*: $F_{1,54} = 0.19$, p = 0.67 ***G*: $\mathbf{F_{1,54} = 10.30}$, $\mathbf{p = 0.002}$** *TxG*: $F_{1,54} = 0.37$, p = 0.55
BRN-B								
SDMT	59.69 ± 9.09	66.17 ± 6.37	54.93 ± 10.56	60.80 ± 10.13				*T*: $F_{1,54} = 3.81$, p = 0.06 *G*: $F_{1,54} = 3.12$, p = 0.08 *TxG*: $F_{1,54} = 0.48$, p = 0.49
PASAT (%)	76.22 ± 8.87	78.33 ± 17.45	74.86 ± 18.18	83.44 ± 13.01				*T*: $F_{1,54} = 1.40$, p = 0.24 *G*: $F_{1,54} = 0.17$, p = 0.68 *TxG*: $F_{1,54} = 0.51$, p = 0.48
SRT Long-Term Storage	58.46 ± 8.08	52.67 ± 12.94	52.07 ± 13.53	52.53 ± 10.29				*T*: $F_{1,54} = 0.60$, p = 0.44 *G*: $F_{1,54} = 0.89$, p = 0.35 *TxG*: $F_{1,54} = 0.82$, p = 0.37
SRT Consistent Long-Term Retrieval	51.31 ± 11.76	47.83 ± 5.71	43.21 ± 14.75	42.07 ± 12.32				*T*: $F_{1,54} = 0.31$, p = 0.58 *G*: $F_{1,54} = 2.82$, p = 0.10 *TxG*: $F_{1,54} = 0.08$, p = 0.78
SRT Delayed Recall	10.23 ± 1.92	10.50 ± 1.98	9.50 ± 2.85	10.27 ± 1.98				*T*: $F_{1,54} = 0.55$, p = 0.46 *G*: $F_{1,54} = 0.48$, p = 0.49 *TxG*: $F_{1,54} = 0.13$, p = 0.72
SPART Long-Term Storage	20.62 ± 6.64	23.33 ± 3.26	20.57 ± 5.06	20.33 ± 5.18				*T*: $F_{1,54} = 0.55$, p = 0.46 *G*: $F_{1,54} = 0.83$, p = 0.37 *TxG*: $F_{1,54} = 0.78$, p = 0.38
SPART Delayed-Recall	7.08 ± 2.72	8.67 ± 1.75	7.07 ± 2.12	7.27 ± 1.83				*T*: $F_{1,54} = 1.75$, p = 0.19 *G*: $F_{1,54} = 1.08$, p = 0.30 *TxG*: $F_{1,54} = 1.07$, p = 0.31
WLGT	22.54 ± 3.57	25.17 ± 3.66	21.14 ± 6.29	21.40 ± 5.58				*T*: $F_{1,54} = 0.82$, p = 0.37 *G*: $F_{1,54} = 2.63$, p = 0.11 *TxG*: $F_{1,54} = 0.55$, p = 0.46
BDI	7.85 ± 5.65[C, D]	4.50 ± 5.24[C, D]	14.21 ± 7.98	11.47 ± 8.33				*T*: $F_{1,54} = 1.84$, p = 0.18 ***G*: $\mathbf{F_{1,54} = 8.83}$, $\mathbf{p = 0.005}$** *TxG*: $F_{1,54} = 0.02$, p = 0.89
FSS		-		47.36 ± 16.01	40.80 ± 17.98	$t_{27} = 1.03$, p = 0.310		
Matrix Subtest (WAIS III)	105.71 ± 14.79	106.43 ± 16.34	111.15 ± 7.95	106.33 ± 12.17				*T*: $F_{1,54} = 0.34$, p = 0.56 *G*: $F_{1,54} = 0.57$, p = 0.45 *TxG*: $F_{1,54} = 0.61$, p = 0.44

HCu: HC untrained group; HCt: HC trained group; MSu: MS untrained group; MSt: MS trained group; Educational level: 1 = Primary education, 2 = Lower secondary education, 3 = Upper secondary education, 4 = Post-secondary education non-tertiary, 5 = First stage of tertiary education, 6 = Second stage of tertiary education; EDSS: Expanded Disability Status Scale; BPF: Brain Parenchymal Fraction; BRN-B: The Brief Repeatable Battery of Neuropsychological Test; SDMT: Symbol Digit Modalities Test; PASAT: Paced Auditory Serial Addition Test; SRT: Selective Reminding Test; SPART: Spatial Recall Test; WLGT: Word List Generation Test. BDI: Beck Depression Inventory; FSS: Fatigue Severity Scale.

[A] denotes statistically significant different from the *HCu group.*
[B] denotes statistically significant different from the *HCt group.*
[C] denotes statistically significant different from the *MSu group.*
[D] denotes statistically significant different from the *MSt group.*

classification accuracy (percent of correctly classified cases) was calculated with the *lda* function of the *MASS* package (Venables and Ripley, 2002) for RStudio.

In addition, Pearson's *r* correlation index was used to assess the strength of the relationship between the degree of change in FC scores (Δ-FC scores) and the degree of change in the number of CRs or RT (Δ-CR and Δ-TR, respectively). The amount of variance in Δ-CR or Δ-TR attributable to the collective changes in Δ-FC was estimated in terms of R^2_{adj} and deviance explained, calculated in appropriate General Additive Models with the *gam* function of the *mgcv* package for Rstudio (Wood, 2004). The resulting effect sizes were characterized as "small", "medium", or "large", according to the benchmarks proposed by Cohen (1988) for R^2 estimates.

3. Results

3.1. Demographic and neuropsychological variables

Table 1 displays the demographic and clinical characteristics of each group of participants. Statistically significant differences between groups were only observed for BDI scores and, as expected, for BPF volume. Conversely, these four groups did not significantly differ on age, gender, neuropsychological performance, or fatigue scores.

3.2. N-back performance

Table 2 shows the descriptive statistics (means and standard deviations) of each group for the number of CRs and RTs during the execution of the 2- and 3-back tasks inside the scanner at S1 and S2.

Regarding the number of CRs during the 2-back performance, a mixed two-way ANOVA (group x training x session) only revealed a main effect of session [S2 > S1; $F_{session}(1, 54) = 16.119, p = 1.85^{-3}, \eta^2 = 0.082$), although the effects of training and the training x session interaction also approached statistical significance [$F_{training}(1, 54) = 2.821$, p = 0.099; $F_{training\ x\ session}(1, 54) = 2.933$, $p = 0.092$). These effects were more evident for RT, where not only the main effects of training and session reached statistical significance, but also their interaction [$F_{training\ x\ session}(1,54) = 21.30$, $p = 2.46^{-4}$, $\eta^2 = 0.059$]. Subsequent post-hoc comparisons revealed that trained participants (MSt and HCt) exhibited shorter RTs in S2 (T < U, $p = 3^{-5}$, $d = -1.36$) than in S1 (T≈U, $p > 0.05$).

Cognitive training also improved 3-back performance. Regarding the number of CRs, a mixed two-way ANOVA (group x training x session) yielded a statistically significant effect of session (p < 0.001) and the

Table 2
Correct Responses (CRs) and Reaction Times (RTs) during the execution of 2 and 3 -back tasks. Differences between groups were analysed using Analysis of Variance (ANOVA) and are described in the text (behavioural fMRI results).

2- back				
	CRs		RTs	
	S1	S2	S1	S2
HCu	13.53 ± 3.37	14.66 ± 2.84	619.37 ± 103.33	570.30 ± 104.63
HCt	14.42 ± 2.90	16.71 ± 2.05	625.72 ± 155.27	467.61 ± 104.41
MSu	14.64 ± 3.73	15.28 ± 2.25	708.46 ± 129.91	707.37 ± 141.62
MSt	4.40 ± 1.76	.53 ± 1.99	606.91 ± 115.75	482.77 ± 79.57
3 -back				
	CRs		RTs	
	S1	S2	S1	S2
HCu	13.13 ± 3.99	12.60 ± 4.17	674.93 ± 144.92	609.09 ± 109.46
HCt	11 .00 ± 3.55	16.50 ± 1.78	640.30 ± 162.14	451.34 ± 90.59
MSu	13.14 ± 3.32	13.28 ± 2.78	737.95 ± 142.51	723.42 ± 138.63
MSt	11.20 ± 4.64	16.33 ± 1.44	629.90 ± 154.05	471.58 ± 118.75

HCu = Healthy controls untrained group; HCt = Healthy controls trained group; MSu = multiple sclerosis unstrained group, MSt = multiple sclerosis trained group, S1 = session one (pre-training); S2 = session 2 (post-training).

training x session interaction [$F_{\text{training x session}}$ (1, 54) = 39.424, $p = 6.07^{-8}$, $\eta^2 = 0.150$]. As the follow-up comparisons show, this interaction effect was due to the higher number of CRs observed in trained participants in S2 (T > U, $p = 1.03^{-5}$, $d = 1.27$), despite the lack of a statistically significant difference between trained and untrained participants in S1 (T≈U, $p > 0.05$). The same pattern of results was observed for RT. In this case, the training and session factors and the training x session interaction reached statistical significance [$F_{\text{training x session}}$ (1, 54) = 23.321, $p = 1.17^{-5}$, $\eta^2 = 0.06$]. Again, this interaction effect was due to the shorter RTs exhibited by trained participants in S2 (T<U, $p = 3.72^{-8}$, d = -1.67), but not in S1 (T ≈ U, $p > 0.05$).

3.3. Between-group differences in FC

Table 3 and Fig. 1 report all the statistically significant effects obtained in the mixed two-way ANOVAs (group x training x session) comparing the FC scores of the four participant groups during the 2- and 3-back tasks. Similarly, to what was observed for the n-back performance indexes (CRs and RT), between-group differences in FC scores arose from training x session interactions, but they were largely independent of the group factor (HC vs MS).

Thus, during the 2-back performance, the only statistically significant effect involving the group factor was a group x training x session interaction in the FC between the left insula and the left precentral gyrus. Follow-up analyses revealed that this effect was solely due to differences between the two groups of MS patients. Moreover, trained participants (HCt and MSt) exhibited stronger FC than untrained participants (HCu and MSu) in several fronto-parietal areas in S2, but not in S1 (training x session interaction, see Table 3). Specifically, trained groups showed increased FC between the left precentral gyrus and left insula, left precuneus and left precentral gyrus, between the left insula and right inferior frontal gyrus, and between the left superior parietal lobe and left precuneus. The size of these univariate differences added up to a multivariate $D = 1.50$ [95%CI: 0.7, 1.94], a small degree of overlap of the Δ-FC score distributions of trained/ untrained participants (45.2%), and a large PS for trained participants (0.86). Accordingly, Δ-FC scores allowed us to correctly identify trained/ untrained participants in 81.03% ([95%CI: 0.69, 0.90], $p = 1.016^{-6}$) of the cases.

Similar between-group differences were observed in FC during 3-back performance. Thus, in trained participants (HC and MS), stronger FC was observed between the left precuneus and left superior parietal lobe and the reverse contrast, as well as between the left insula and right inferior frontal gyrus (see Table 3 for further details). These univariate differences added up, yielding a $D = 1.15$ [95%CI: 0.44, 1.58], which translated into a degree of multivariate overlap of the Δ-FC score distributions of trained/ untrained participants equal to 56.6%, and to a moderate-to-large PS for trained participants (0.79). These Δ-FC scores led to 79.31% ([95%CI: 0.67, 0.89], $p = 4.11^{-6}$) of cases being correctly classified.

No other statistically significant differences between groups were observed.

Table 3
Between groups differences in functional connectivity (FC) during 2 and 3 -back tasks. The table displays the statistically significant ($p < 0.05$, FWE-corrected) ANOVA effects for FC scores. L = left; R = Right; T = trained groups (all trained participants that included MSt and HCt); U = Untrained participants (all untrained participants that included MSu and HCu); S1 = session one (pre-training); S2 = session 2 (post-training).

2 -back Anatomical regions	**Training x Session effects**		**S1**	**S2**
L precentral gyrus-L insula	F(1,54) = 10.91, p = 0.032, η^2 = 0.07	T ≈ U	T>U p>0.05	p = 0.016, d = 0.65
L insula-R inferior frontal gyrus (triangularis)	F(1,54) = 9.71, p = 0.048, η^2 = 0.07	T ≈ U	T>U, p>0.05	p = 0.035, d = 0.57
L precuneus-L precentral gyrus	F(1,54) = 13.88, p = 0.007,η^2 = 0.09	T<U	T>U p = 0.014, d = -0.66	p = 0.047, d = 0.53
L superior parietal lobe-L precuneus	F(1,54) = 10.47, p = 0.035,η^2 = 0.11	T ≈ U	T>U p>0.05	p = 0.003, d = 0.83
Group x Training x Session effects		**S1**	**S2**	
L insula-L precentral gyrus	F(1,54) = 10.75, p = 0.032, η^2 = 0.08	MSu>MSt	MSt>MSu p = 0.013, d = -0.99 p = 0.005, d = 1.13	
3 -back Anatomical regions	**Training x Session effects**		**S1**	**S2**
L precuneus-L superior parietal lobe	F(1,54) = 11.31, p = 0.016,η^2 = 0.10	T ≈ U	T>U p>0.05	p = 0.002, d = 0.83
L superior parietal lobe-L precuneus	F(1,54) = 10.44, p = 0.032, η^2 = 0.09	T ≈ U	T>U p>0.05	p = 0.01, d = 0.69
L insula-R inferior frontal gyrus (triangularis)	F(1,54) = 9.82, p = 0.048,η^2 = 0.08	T ≈ U	T>Up>0.05	p = 0.006, d = 0.75

3.4. Relationships between FC and n-back performance

As Fig. 2 shows, the training-induced changes (S2 -S1 difference) in FC and in the n-back performance indexes (CRs and RT) were related.

Regarding 2-back performance, the S2-S1 change in the number of CRs (Δ-CR) was directly and significantly correlated with the Δ-FC between the left precuneus and the left precentral gyrus. All the other statistically significant FC changes were unrelated to the Δ-CR. Thus, the combination of all these Δ-FC scores in a single predictive model explained a relatively low amount of the variance in Δ-CR (deviance explained = 10.4%; adjusted $R^2 = 0.090$; "small" effect size). On the other hand, the S2-S1 change in the RT (Δ-RT) was inverse and significantly correlated with the Δ-FC between the left precentral and the left insula, between the left insula and the right inferior frontal gyrus, and between the left superior parietal lobe and the left precuneus. The Δ-FC between the left precuneus and the left precentral was only marginally

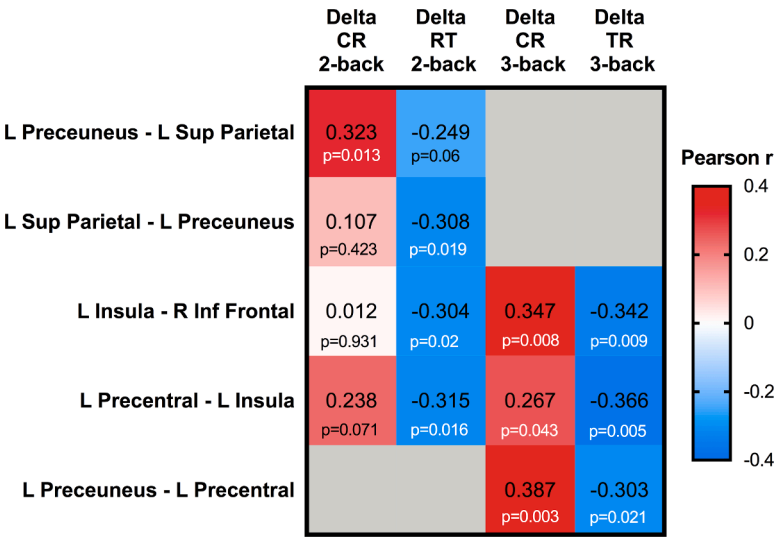

Fig. 2. Relationship between training-induced changes in functional connectivity (FC) and in n-back performance. The figure displays the values of Pearson's *r* correlation index quantifying the relationship between S2 (post training)-S1(pre training), the gains in performance (delta correct responses -CR- and delta reaction times -RT-), and the S2-S1 changes in FC summarized in Table 3. The *r* values and their associated *p*-values are reported inside the cells. Cells coloured in red and blue tones illustrate direct and inverse correlations, respectively. Gray coloured cells denote FC paths that did not reach statistical significance on either the 2-back or 3-back task. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

correlated ($p = 0.06$) with the Δ-RT. Taken together, these FC changes accounted for a large amount of Δ-RT variance (deviance explained = 23.6%, adjusted $R^2 = 0.18$; "medium" effect size). Finally, although it is not shown in Fig. 1, the Δ-FC between the left insula and the left precentral gyrus observed in MS patients was inversely correlated with their Δ-RT ($r = -0.44$, $p = 0.016$).

Regarding 3-back performance, the S2 vs. S1 changes in the number of CRs were directly and significantly correlated with the Δ-FC between the left insula and the right inferior frontal gyrus, between the left superior parietal lobe and the left precuneus, and between the left precuneus and the left superior parietal lobe. In this case, the deviance in Δ-CR explained by the Δ-FC scores was 24.4% (adjusted $R^2 = 0.22$; "medium" effect size). In addition, these Δ-FC scores also showed inverse and statistically significant correlations with the S2-S1 RT changes. Combining these Δ-FC scores into a single predictive model allowed us to account for 34.6% of the deviance in Δ-CR (adjusted $R^2 = 0.29$; "medium" effect size).

4. Discussion

This study reveals the benefits of specific and intensive computerized WM training in a group of cognitively preserved MS patients and HC. First, we observed that, after four days (60 min/ day) of intensive training specifically focused on WM, both MS patients and HC improved their WM performance, exhibiting greater CR rates and lower RTs. Second, we observed that the same training program led to an increase in neural FC in the fronto-parietal network belonging to WM. In addition, we also observed that the two kinds of training-induced changes (better performance and stronger FC) were associated with each other.

Compared to the non-trained participants, trained participants (HC and MS) showed enhanced n-back performance in S2 compared to S1. These effects were observed as an increase in the CR rate (which provides a purer measure of WM) and as a RT decrease (which might be interpreted as reflecting an enhancement of IPS). These results confirm and extend those from other previous studies using this specific WM training program (Aguirre et al., 2019; Covey et al., 2018; Thompson et al., 2016).

As expected, cognitive training resulted in increased FC during the execution of the 2- and 3-back tasks. With the only exception of a selective enhancement of the FC between the left insula and left precentral gyrus observed in trained MS patients (but not in HC) during 2-back (but not 3-back) performance, the effects of training on FC were quite similar in all the trained participants. Thus, trained HC and trained MS patients exhibited increased FC between superior parietal areas (including the precuneus) and between parietal and frontal areas (including the precentral gyrus, the inferior frontal gyrus, and insula). The precuneus is involved in shifting attention processes, especially when the targets objectives are presented in different locations in the space and required motor responses (Cavanna and Trimble, 2006). The left precentral gyrus (that comprises part of the primary motor cortex) is related to hand movement and, as we also observed in the present study, its activity had been previously associated to lower RTs in WM tasks (Emch et al., 2019). On the other hand, the insula has an important function in multimodal sensory processes and it has a similar role to that of the precentral gyrus in hand, but also, in eye movement and language processing (Oh et al., 2014; Wang et al., 2019). Moreover, the left insula is part of the articulatory loop and, as such, it is considered as having an essential functional role in WM tasks (Emch et al., 2019). Finally, the inferior frontal gyrus plays an important role in inhibitory processes, which are necessary to suppress context-inappropriate responses and adequately perform WM tasks (e.g. to avoid reporting the stimulus presented in the 2- and 3-back tasks (Hampshire et al., 2010; Levy and Wagner, 2011)). These FC changes conformed a clearly defined pattern that made it possible to correctly distinguish trained and non-trained participants in ≈80% of the cases. Interestingly, these training-induced S2-S1 FC increases were directly correlated with the S2-S1 gains in CR rates and inversely related to the S2-S1 RT changes. In fact, these Δ-FC scores additively explained up to 24.4% and 29% of the variance in the Δ-CR and Δ-RT, respectively.

Taken together, the results of the present study confirm and extend previous findings showing that cognitive training leads to enhanced FC and improved task performance in MS patients (Bonavita et al., 2015; De Giglio et al., 2016; Leavitt et al., 2014; Parisi et al., 2014b). Moreover, and in agreement with previous studies conducted in healthy volunteers (Constantinidis and Klingberg, 2016), we observed that a WM training increased FC between fronto-parietal areas and this FC increase was significantly correlated to the training-induce performance improvements.

Although the present results are encouraging and confirm the efficacy of training to stimulate brain plasticity mechanisms that can enhance cognitive performance in MS patients, the present study also has some limitations that should be considered. First, as in most studies that assess the effects of cognitive training programs in MS patients, the recruited sample was small. Therefore, the statistical power achieved might be suboptimal, and it is likely that this study failed to identify all the relevant between-group differences in FC.

Second, we recruited homogeneous groups of participants. Specifically, all of the MS patients were diagnosed with the RR phenotype,

presented a few years of disease evolution, and were cognitively preserved (the neuropsychological performance of these patients was indistinguishable from that of the HC, and no statistically significant differences between MS and HC were found on any cognitive test). Although the use of homogenous groups reduces spurious variability, partially counteracts the negative effects of the reduced sample size, and facilitates the identification of between-group differences, it also reduces the generalizability of the results (Carter et al., 2015). Thus, we cannot conclude that this training protocol would improve WM and IPS to the same degree in MS patients with different clinical characteristics, particularly those with severe cognitive impairment. Similarly, the present study does not provide information about a possible moderating role of potentially relevant variables (e.g. age, gender, cognitive reserve, atrophy, etc.) in the beneficial effects of this cognitive training program.

Third, in the present study, the possible long-term effects of our WM training program were not examined. In this regard, future studies should determine to what extent the observed FC changes and task performance enhancement persist over time.

Finally, WM capabilities largely underlie and subserve other cognitive abilities, and as previously suggested (Covey et al., 2018; Mitolo et al., 2015), the training-induced WM improvements could lead to improvements in other cognitive domains. However, although we observed a reduction in RT that might be interpreted as being the result of IPS improvement, we did not specifically test these possible generalization or transfer effects to other cognitive processes. Future studies should specifically assess the extent to which WM training improves other cognitive functions and other important cognitive rehabilitation goals in MS patients, such as perceived quality of life or emotional/mood status. Furthermore, in this study we used and intensive training, that only included four consecutive days. In this sense, future studies should explore the efficacy of training programs with different intensities/duration which could be beneficial for groups of patients with a higher degree of cognitive impairment (moderate/ severe).

In conclusion, this study reinforces the notion that short periods of cognitive training could be useful to improve cognitive functions and brain resources in patients with brain damage. More specifically, implementing early interventions in MS patients, could be an useful strategy to prevent or attenuate the cognitive decline in diagnosed MS patients (Covey et al., 2018; Lampit et al., 2019).

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Authors' contributions

C.F and C.A contributed to the planning and preparation of the current study and also conceived the experimental design. A.M-P, E.B and C.A prepared the paradigm. The patients were selected by N.A and J. C-G. A.M-P, E.B, N.A, J.C-G R.B-T and SF-E were responsible for collecting the fMRI data. C.S-S and C.F performed the statistical analysis and contributed to the interpretation of the results. C.F, N.A and C.S-S generated the manuscript. C.F, C.S-S and N.A were intensely involved in the final version of the manuscript. All authors read and approved the final manuscript.

Credit Author Statement

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.msard.2021.102976.

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