Functional MRI correlates of cognitive performance in patients with a clinically isolated syndrome suggestive of MS at presentation: an activation and connectivity study

Journal:	*Multiple Sclerosis Journal*
Manuscript ID:	Draft
Manuscript Type:	Original Research Paper
Date Submitted by the Author:	n/a
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Keywords:	MRI, Multiple sclerosis, T2 lesions
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	Addition Test (PASAT). According to their PASAT performance, CIS patients were divided into two groups: 10 with a low PASAT performance (< 1SD from the mean value of HC) were considered "cognitive impairment" (CI); 8 patients were defined as "cognitively preserved" (CP). Between-group differences in the patterns of brain activations and effective connectivity were assessed. Results. During PASAT, compared to HC, CIS patients showed increased activations of the bilateral inferior parietal lobe (IPL), bilateral precuneus, bilateral middle frontal gyrus (MFG), left anterior cingulate cortex (ACC), left claustrum, right thalamus and right caudate nucleus. When CIS patients were analyzed, the CI group had a more significant activation of the bilateral IPL than HC and CP patients. Compared to CP patients, they also had more significant recruitment of the right superior parietal lobe, right cerebellum, left MFG and left ACC. The analysis of effective connectivity showed stronger connections between several regions of the right hemisphere involved in working memory function in CI patients vs. CP and HC. Conclusions. The observed differences in the patterns of cortical recruitment and connectivity in CI patients during PASAT performance are likely to be adaptive and have a role in limiting cognitive dysfunctions early in the course of MS.

Functional MRI correlates of cognitive performance in patients with a clinically isolated syndrome suggestive of MS at presentation: an activation and connectivity study

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Abstract

Objective. To assess whether abnormalities on functional magnetic resonance imaging (fMRI) are related to cognitive function in patients at presentation with clinically isolated syndrome (CIS) suggestive of MS.

Methods. Eighteen CIS patients and 15 healthy controls (HC) performed an adapted fMRI version of the Paced Auditory Serial Addition Test (PASAT). According to their PASAT performance, CIS patients were divided into two groups: 10 with a low PASAT performance (< 1SD from the mean value of HC) were considered “cognitive impairment” (CI); 8 patients were defined as “cognitively preserved” (CP). Between-group differences in the patterns of brain activations and effective connectivity were assessed.

Results. During PASAT, compared to HC, CIS patients showed increased activations of the bilateral inferior parietal lobe (IPL), bilateral precuneus, bilateral middle frontal gyrus (MFG), left anterior cingulate cortex (ACC), left claustrum, right thalamus and right caudate nucleus. When CIS patients were analyzed, the CI group had a more significant activation of the bilateral IPL than HC and CP patients. Compared to CP patients, they also had more significant recruitment of the right superior parietal lobe, right cerebellum, left MFG and left ACC. The analysis of effective connectivity showed stronger connections between several regions of the right hemisphere involved in working memory function in CI patients vs. CP and HC.

Conclusions. The observed differences in the patterns of cortical recruitment and connectivity in CI patients during PASAT performance are likely to be adaptive and have a role in limiting cognitive dysfunctions early in the course of MS.

Introduction

In about 85% of patients with multiple sclerosis (MS), the clinical onset of the disease is a clinically isolated syndrome (CIS), involving the optic nerve, brainstem or spinal cord. Approximately 50-80% of these patients already have visible lesions on magnetic resonance imaging (MRI) scans, consistent with prior disease activity [1].

It is known that cognitive deficits play an important role in MS and that they impact significantly on patient activities of daily living and quality of life [2]. However, relatively little is known about the prevalence of cognitive impairment at the onset of the disease in CIS patients. Recent studies in this clinical population have described the presence of cognitive deficits ranging from mild to moderate, depending on the criteria used to define such an impairment [3,4,5,6].

Cognitive dysfunction in MS involves several domains including information processing speed, attention, memory and frontal executive functions [7]. This heterogeneity is usually not observed in individuals with CIS, in whom only deficits of information processing speed and working memory have been described [5]. A characterization of the cognitive profile of CIS patients as well as the mechanisms underlying the preservation of some cognitive functions in this condition might improve our understanding of the development of cognitive impairment in MS. Indeed, it has been recently demonstrated that cognitive deficits in CIS patients have a prognostic value to predict subsequent conversion to definite MS [8].

In relation to this, a valuable tool is represented by functional MRI (fMRI). In the last few years, fMRI studies have disclosed differences in the patterns of brain recruitment between MS patients and healthy controls (HC) when performing various active tasks. Some of these studies have suggested that, at least in some cases, an altered pattern of brain recruitment might contribute to limit the clinical consequences of tissue damage

[9,10,11]. Brain reorganization has also been observed in CIS patients when performing cognitive [12] and motor tasks [13]. To better characterize differences in the patterns of brain activations between MS patients and HC, a few studies have also analyzed functional/effective connectivity among different brain regions [14,15,16,17,18]. However, only a seminal study has shown an increased connectivity of brain regions of the working memory network in CIS compared to HC [19].

To examine possible early fMRI cognition-associated abnormalities, we studied a sample of CIS patients using an adapted version of the Paced Auditory Serial Addition Test (PASAT), which has been previously applied in several fMRI studies of patients with definite MS [9,10,12]. This task is sensitive to abnormalities of working memory and information processing speed. In addition, to characterize further the possible differences in brain recruitment between CIS patients and HC, we also run an effective connectivity analysis to obtain a more complete picture of the functional abnormalities associated to cognitive performance in these patients.

Materials and methods

Participants

Eighteen right-handed patients at presentation with CIS suggestive of MS and paraclinical evidence of dissemination in space [20] were recruited consecutively at the Hospital Universitari La Fe and the Hospital Clinic Universitari MS Unit, Valencia, Spain. All patients were examined within 3 months from the first clinical episode and were free of steroids treatment for at least one month. Alternative neurological diseases were excluded by appropriate investigations [21, 22]. Exclusion criteria were concomitant therapy with antidepressants, psychoactive drugs, and a history of major medical, neurological or psychiatric disorders as well as drug and alcohol abuse. Depression was excluded with the

Beck Depression Inventory (BDI). There were 10 women and eight men, and their mean age was 32.9 years (range = 18-47 years). Oligoclonal bands were found in the cerebrospinal fluid of all patients. Disability was rated using the Expanded Disability Status Scale (EDSS) Score [23]. Fifteen aged-matched right-handed individuals with no history of neurological or psychiatric diseases served as controls.

Neuropsychological assessment

Following neurological assessment and prior to the MRI acquisition, all participants were assessed by an experienced neuropsychologist, blinded to clinical and MRI findings. This assessment included the Brief Repeatable of Neuropsychological test (BRB-N) validated in a Spanish population [24], the Matrix subtest included in the Wechsler Adult Intelligence Scale III Battery (WAIS III) [25], which was used to match groups in term of their intelligence quotients (IQ).

MRI acquisition

Using a 3.0 Tesla scanner (Siemens Trio, Siemens, Erlangen, Germany), the following sequences of the brain were acquired from all participants: 1) axial T2-weighted fast spin echo (TR/TE = 6740/97; ETL = 15; FOV = 250 × 250 mm; matrix size = 256 × 256; 40 contiguous slices; slice thickness = 3 mm); 2) axial echo-planar imaging (TR/TE = 3000/50 ms; flip angle = 90°; FOV = 250 × 250 mm; matrix size = 64 × 64; 29 contiguous slices, slice thickness = 4.5 mm) during PASAT task.

Experimental Design

Participants were instructed to perform the fMRI-adapted auditory PASAT task [10]. PASAT consisted of a block design paradigm with 6 blocks of 1 min each: 3 for the control condition and 3 for the active condition. In the PASAT task, a 1-digit number between 1 and 9 was randomly presented every 3 s. During the control condition, participants were instructed to repeat the last number heard. During the active condition,

participants performed the PASAT task, which involved adding up the last two numbers heard. Subjects were asked to give overt verbal responses, which were recorded by an observer inside the scanner room. The stimuli for the tasks were presented using fMRI-compatible headphones (VisuaStim, Resonance Technologies, Inc). The sound volume was adjusted so that each participant could hear the stimuli of the PASAT test properly. Before fMRI was performed, all subjects underwent a 10-minute practice session, which included different stimuli presented in the scanner. Foam cushioning was used to immobilize their head within the coil in order to minimize motion artifacts.

Statistical analysis of clinical and cognitive data

Statistical analysis was performed using the Statistical Software Package (Statsoft, Inc). Inter-group comparisons were made in two steps. First, HC and CIS patients were compared in term of their neuropsychological performance. This analysis showed no difference between the two groups. As a second step, and as previously described [12], CIS patients were divided into two groups according to their performance at PASAT, based on the strategy suggested by Sepulcre et al. [24]: patients with PASAT performance 1SD below the mean of HC were considered as cognitively impaired (CI) (n = 10). One-way ANOVAs and the Fisher LSD post-hoc test were used to analyze the differences among the three groups for clinical and cognitive data.

MRI analysis

All MRI postprocessing was performed by a single experienced observer, who was unaware of subjects' identity. T2 lesions were identified and T2 lesion load (LL) measured using the Jim software (Version 4.0, Xinapse Systems, Northants, UK, http://www.xinapse.com).

fMRI data were analyzed using the Statistical Parametric Mapping (SPM5) software (Welcome Department of Cognitive Neurology, London, UK). Prior to the statistical

analysis, all images were reoriented according to the anterior-posterior commissural line, realigned to the first one, spatially normalized into the Montreal Neurological Institute (MNI) space, and smoothed with an 8 mm Gaussian kernel filter. SPM calculation was based on the General Lineal Model [26]. The motion parameters estimated during realignment were included in the statistical analysis as regressors. First-level task-related contrast images were created for each subject and then used in a second-level random-effect analysis (one-sample t-test) to define the brain patterns of recruitment in each group separately (controls and CIS patients), using a threshold of $p< 0.001$ family-wise error (FDR) corrected, and a minimum cluster extent (k) of 20 contiguous voxels. Between-group comparisons were assessed using a one-way ANOVA, with a threshold for significance set at $p<0.05$ FDR corrected, with a minimum cluster extent (k) of 20 contiguous voxels.

Effective connectivity analysis

DCM is a mathematical framework which allows to characterize effective connectivity, defined as the causal influence that one brain region exerts over another one [27]. It requires defining an *a priori* model, usually chosen among possible alternative models using a Bayesian model selection procedure [28]. In our case, this model was established based on brain areas known to be related to working memory, as shown by several previous fMRI studies [9,10,12]. The areas selected were: the left (L) and right (R) middle third of the middle frontal gyrus (MFG), the L and R inferior frontal gyrus (IFG), the L and R anterior cingulate cortex (ACC), and the L and R inferior parietal lobe (IPL). Using the VOI toolbox of SPM5, time series (adjusted for the effect of interest) were extracted from a spherical volume (5 mm radius) centered at the most significant voxel within each cluster of the SPMt map, displaying in each subject the brain areas active

during the PASAT task at p<0.001, uncorrected. We assumed that the effect of the task entered the network via the activation of the L MFG.

The Bayesian model selection [28] was performed to test which of the two possible DCM models, including the previous areas, fitted better the data of the two study groups. The first DCM model (M1) assumed a bilinear interconnection between all the above mentioned areas. The second (M2) was identical to the model previously proposed by Au Duong et al. [19], and considered only the connections shown in Figure 1 as possible connections. The comparison of the two models showed that the M2 model was superior to M1 in both HC (Bayesian factor [BF] of M1 = $9.001*10^{-66}$ *vs*. BF of M2 = $1.111*10^{65}$) and CIS patients (BF of M1 = $1.705*10^{-11}$ *vs*. BF of M2 = $1.132*10^{112}$). As a consequence, the intrinsic connectivity coefficients of M2 were estimated for each subject using a Bayesian approach.

A multivariate analysis of variance (MANOVA) was used to examine inter-group differences in the strength of connectivity of the previously estimated DCM coefficients.

Results

Demographic, clinical, neuropsychological and conventional MRI findings

Table 1 summarizes the main demographic, clinical and conventional MRI findings from CIS patients as well as their performance at PASAT. Table 2 presents the main demographic, neuropsychological, and conventional MRI characteristics of the three study groups.

EDSS scores (p = 0.001) and T2 LL (p = 0.03) were significantly different between CP and CI patients. CI patients did more errors at the behavioral PASAT task compared to HC (p = 0.04) and CP patients (p = 0.001). Similar results were obtained when analyzing the fMRI PASAT test (p = 0.04 *vs*. HC, and p = 0.002 *vs*. CP patients).

fMRI findings

a) Within-group activations. The results of within-group activations during the PASAT are summarized in Table 3 and illustrated in Figure 2. Both HC and CIS patients showed a recruitment of several areas in the frontal and parietal lobes, bilaterally, as well as areas that are part of the working memory network, bilaterally.

b) Between-group comparisons. During PASAT, compared to HC, CIS patients experienced a more significant recruitment of the bilateral IPL, bilateral precuneus, bilateral MFG, left ACC, left claustrum, right thalamus and right caudate nucleus. When CIS patients were analyzed according to their PASAT performance, the CI group had a more significant activation of the bilateral IPL than HC and CP patients. Compared to CP patients, they also had a more significant activation of the right superior parietal lobe, right cerebellum, left MFG and left ACC (Table 4, Figure 3). No areas were significantly more activated in CP patients *vs*. HC and CI patients.

c) Analysis of effective connectivity. Inter-group differences in the strength of connectivity coefficients are summarized in Table 5 and Figure 1. Compared to both HC and CP patients, CI patients had an increased connectivity between the: 1) R IFG and R IPL ($p = 0.002$ *vs*. HC, and $p = 0.0005$ *vs*. CP patients), 2) L IPL and R IPL ($p = 0.008$ *vs*. HC, and $p = 0.0005$ *vs*. CP patients), and 3) R IPL and R IFG ($p = 0.003$ *vs*. HC, and $p = 0.01$ *vs*. CP patients). Compared to CP, CI patients also experienced an increased connectivity between the: R ACC and R IPL ($p = 0.009$), and R MFG and R IFG ($p = 0.01$).

Discussion

This study extends our knowledge about the functional changes which can occur in patients at presentation with a CIS suggestive of MS. To detect subtle changes in cognitive

functions, we used an adapted version of the PASAT, a task that assesses information processing speed and working memory, since these functions are known to be altered in these patients [5].

In line with the results of previous studies [9,10,12], when compared to HC, CIS patients showed an increased recruitment of several regions mainly located in the frontal and parietal lobes, bilaterally. All of these regions contribute to different aspects of working memory processing and the majority of them have been demonstrated to have a load-depending activity during working memory tasks [29,30].

Notably, when CIS patients were subdivided into CI and CP according to their PASAT performance, significant differences emerged between the study groups, not only in the patterns of cortical recruitment, but also in the strengths of functional connections among different brain areas. Compared to the other two groups, CI patients experienced an increased recruitment of areas located in the IPL, bilaterally, which are known to be involved in the maintenance and manipulation of a working memory information [31]. Compared to CP patients, they also experienced an additional recruitment of regions located in the fronto-parietal lobes and cerebellum, which is known to be involved in high-level cognitive functions and conflict adaptation [32]. It is worth noting that, in order to be able to detect early markers of cognitive dysfunction in CIS, we defined as CI those patients with 1 SD difference in PASAT performance with respect to the mean of HC (while the cut-off usually applied is 2 or 1.5 SD). Our results indicate that, even in patients with such a subclinical impairment, an increased pattern of cortical recruitment does occur, probably reflecting an adaptive mechanism likely contributing to limit the clinical consequences of disease-related tissue injury.

The analysis of effective connectivity, which was based on the model of brain interconnections for PASAT performance previously proposed by Au Duong et al. [19],

allowed us to characterize better the pattern of fMRI abnormalities in CIS patients. In particular, compared to CP, CI patients experienced stronger connections between the R MFG and R IFG and between the R ACC and R IPL. They also had an increased connectivity between the R IFG and R IPL and between the L and R IPL compared to both CP patients and healthy controls, thus indicating an increased task demand for the R hemisphere in these patients. The notion that an increased recruitment of areas of the R hemisphere has a critical role for cognitive performance of MS patients agrees with previous activation [33,9,34] and connectivity [14] studies performed in patients with CIS and RRMS, as well as with several studies of the motor network (for a review, see Filippi and Rocca [36], which showed that a recruitment of homologous regions of the two hemispheres is one of the main compensatory mechanisms, at least at the earliest clinical phases of the disease. Differently from previous studies, which found abnormalities of brain recruitment in patients without overt clinical impairment [37, 38] our analysis did not reveal any abnormality of activation and connectivity between CP CIS patients and HC. Clearly, we can not exclude that our study was underpowered to detect such a difference, considering the relatively small size of this group.

In this study, we carefully selected a cohort of *de novo* CIS patients that were studied within two months from the diagnosis. Even using such a strict criterion, differences among CIS patients were found using fMRI. This is in line with the results of a previous study of the motor system [38] which showed an increased recruitment of several areas of the motor network in CIS patients assessed within three months from the onset of the clinical symptomatology. Interestingly, compared to CP, CI patients also had a higher EDSS score and T2 lesion load. These results strengthen the notion that CIS patients are a heterogeneous population and call for a comprehensive characterization of functional and structural MRI abnormalities in patients at disease onset also in the perspective of

identifying those patients at a higher risk of evolution to definite MS. Indeed, a seminal study showed that an abnormal pattern of cortical recruitment at disease onset could contribute to identify those CIS patients evolving to definite MS after one year [39].

In conclusion, our observations contribute further to support the notion of plasticity processes in CIS patients, which might counteract the cognitive consequences of diffuse tissue damage and allow, at least temporarily, a preservation of their cognitive capabilities.

Acknowledgments. This research has been supported by the following grants: Alicia Koplowitz Foundation; CSD2007-00012 (Consolider-Ingenio 2010 Programme), SEJ2007-65929/PSIC and PSI2010-20168 from the Spanish Ministry of Science and Education; the Dávalos Foundation Grant and P1·1A2010-07 from Bancaixa.

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Table 1. Individual characteristics of patients at presentation with clinically isolated syndrome (CIS) suggestive of multiple sclerosis (MS).

Patient	Age	Clinical syndrome	EDSS score	T_2 lesion load mm^3	PASAT score
1	41	Hemispheric	0	1495	58
2	23	Spinal cord syndrome	3.5	9628	45
3	23	Spinal cord syndrome	2.0	2153	45
4	34	Hemispheric	0	203	49
5	32	Hemispheric	2.0	220	45
6	25	Spinal cord syndrome	0	336	52
7	28	Hemispheric	0	688	49
8	18	Brainstem syndrome	1.5	1106	56
9	41	Optic neuritis	1.0	615	49
10	45	Optic neuritis	1.5	230	39
11	28	Optic neuritis	1.5	7825	34
12	38	Brainstem syndrome	1.5	1911	39
13	29	Brainstem syndrome	2.0	5367	35
14	31	Brainstem syndrome	1.5	3498	45
15	38	Spinal cord syndrome	2.0	2707	34
16	47	Spinal cord syndrome	1.5	277	49
17	28	Spinal cord syndrome	1.0	379	35
18	43	Optic neuritis	1.0	210	50

List of abbreviations: EDSS=Expanded Disability Status Scale; PASAT=Paced Auditory Serial Addition Test.

Table 2. Main demographic, clinical, neuropsychological and Magnetic Resonance Imaging (MRI) characteristics of the three study groups.

Variable	HC (n=15)	CP patients (n=8)	CI patients (n=10)	Group effect
Mean Age (SD) (years)	32.3 (7.2)	34.9 (10.9)	31.5 (7.01)	F= 0.4, p=0.66
Median EDSS (range)	-	0.5 (0-1.5)	1.75 (1.0-3.5)	-
Educational level (SD) (years)	12.9 (2.5)	12.6 (2.9)	12.5 (2.1)	F= 0.06, p=0.93
Matrix (WAIS III) (SD)	18.6 (1.8)	19.5 (2.7)	18.6 (3.2)	F=0.34, p=0.7
SRT long term storage (SD)	46.6 (9.0)	44.1 (11.2)	52.8 (9.7)	F= 2.91, p=0.06
SRT consistence long term retrieval (SD)	37.1 (8.0)	40.2 (13.2)	42.8 (14.1)	F=0.74, p=0.48
SRT delayed recall (SD)	8.0 (1.4)	8.8 (1.7)	9.4 (1.8)	F=2.02, p=0.14
10/36 SPART long term storage (SD)	21.7 (3.9)	21.5 (5.1)	20.6 (3.4)	F= 0.24, p=0.78
10/36 SPART delayed recall (SD)	8.2 (1.9)	7.7 (2.4)	7.7 (1.3)	F= 0.25, p=0.77
SDMT (SD)	58.9 (10.8)	56.1 (12.3)	49.4 (10.6)	F=2.18, p=0.12
WLGT (SD)	25.6 (7.1)	25.2 (4.4)	22.6 (4.5)	F= 0.85, p=0.43
PASAT 3 seconds (SD)	46.1 (8.4)	51.8 (3.4)	40.2 (4.7)	F=6.62, p=0.004
BDI (SD)	4.1 (1.1)	5.5 (7.6)	5.8 (4.1)	F=0.36, p=0.69
PASAT fMRI correct responses	87.5 (10.5)	96.7 (2.37)	76.3 (19.81)	F=5.53, p=0.009
T2 lesion load mm^3 (SD)	-	615.1 (470.9)	3392.0 (3262.1)	F=5.63, p=0.03

List of abbreviations: HC= Healthy controls, CI=Cognitively impaired; CP=cognitively preserved; EDSS=Expanded Disability Status Scale; WAIS=Wechsler Adult Intelligence Scale III Adult; SRT=Selective reminding test; SPART=Spatial Recall test; SDMT=Symbol digit modalities test; WLGT=word list generation test; PASAT=Paced Auditory Serial Addition Test; BDI=Beck depression inventory; functional Magnetic Resonance Imaging (fMRI).

Table 3. Areas significantly activated during the Paced Auditory Serial Addition Test (PASAT) in Healthy Controls (HC) and Clinically Isolated Syndromes (CIS) patients (one-sample t test in each group; p<0.001, FDR-corrected, k=20).

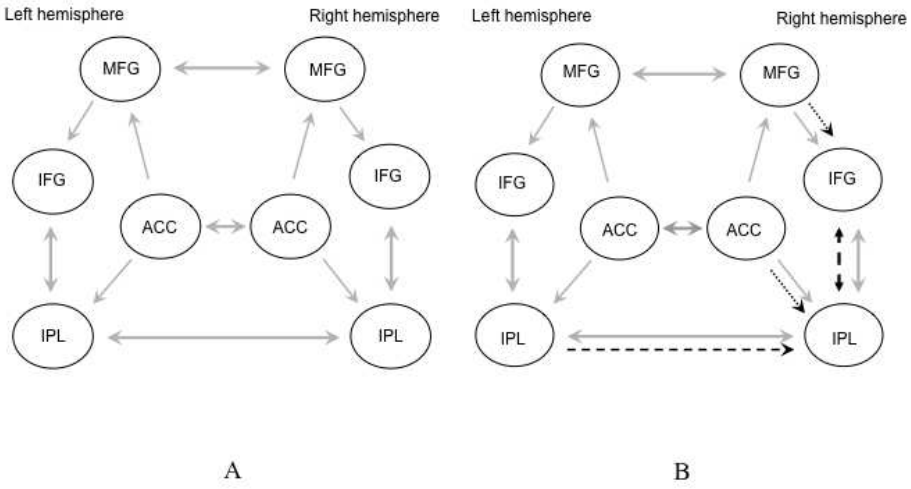
MNI=Montreal Neurologic Institute.

HC				CIS patients			
MNI Coordinates	Anatomical localization	Cluster size	Voxel z score	MNI Coordinates	Anatomical localization	Cluster size	Voxel z score
				9 15 45	Right cingulated gyrus	2917	5.21
-9 12 48	Left medial frontal gyrus	2596	6.16	-9 18 45	Left cingulate gyrus		5.43
-51 15 30	Left middle frontal gyrus		5.89	27 -3 51	Right middle frontal gyrus		4.98
39 -30 -9	Right temporal lobe, subgyral	32	4.79	-6 9 48	Left medial frontal gyrus		5.21
-51 -30 -3	Left middle temporal gryus	47	3.88	-36 -6 42	Left precentral gyrus		5.10
30 -54 36	Right parietal lobe, subgyral	192	4.62	-36 36 21	Left middle frontal gyrus		5.05
				-45 15 6	Left inferior frontal gyrus		4.73
30 -54 48	Right inferior parietal lobe		4.43	18 -54 51	Right parietal lobe, precuneus		5.35
-27 -66 39	Left parietal lobe, precuneus	691	5.84	39 -39 39	Right inferior parietal lobe		5.27
-39 -45 42	Left inferior parietal lobe		5.70	-39 -42 42	Left inferior parietal lobe	948	5.93
27 -69 12	Right occipital lobe, subgyral	53	4.59	-24 -51 42	Left parietal lobe, subgyral		5.64
-30 -63 12	Left occipital lobe, subgyral	21	4.26	-9 -84 0	Left occipital lobe, lingual gyrus	112	4.27
12 -75 -21	Right cerebellum, decline	642	5.09	9 -72 -21	Right cerebellum, decline	352	4.93
-39 -66 -27	Left cerebellum, decline	42	4.47	-33 -63 -30	Left cerebellum, decline	38	4.60

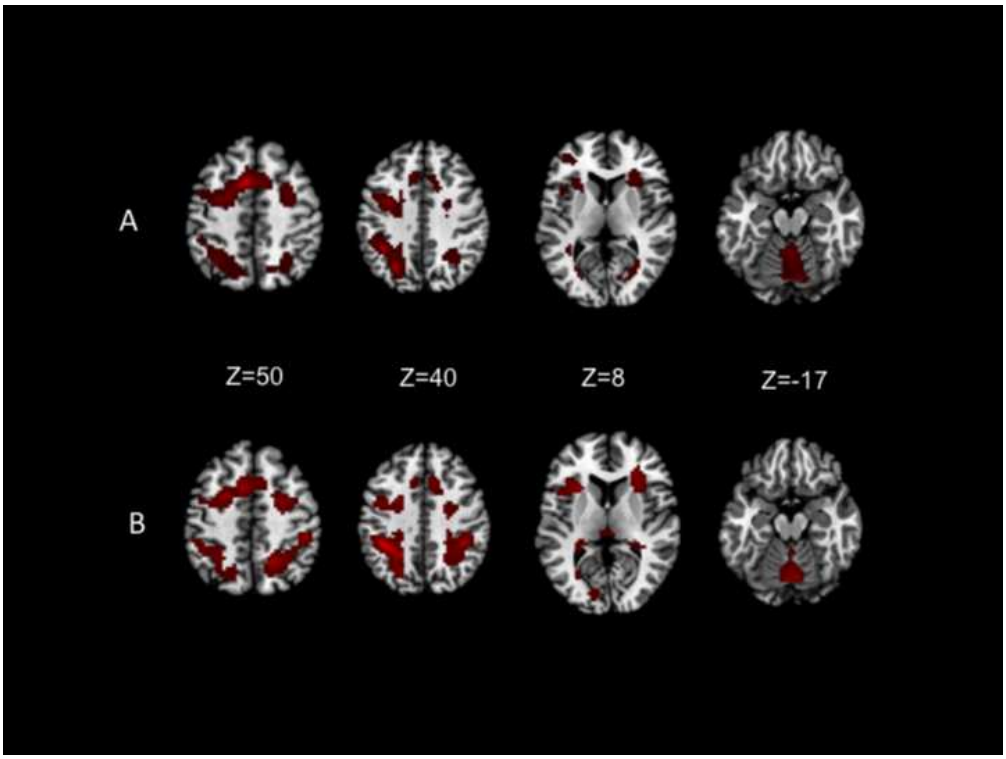
Table 4. Areas showing significant activation differences between groups (p<0.05, FDR-corrected), k=20 voxels.

MNI Coordinates	Anatomical localization	Cluster size	Voxel z score
CIS *vs.* HC			
-39 -42 41	Left inferior parietal lobe	377	5.15
-24 -48 36	Left cingulate gyrus		4.29
-18 -59 47	Left precuneus		3.97
-24 -33 43	Left cingulate gyrus		3.88
-27 -53 52	Left precuneus		3.50
27 -50 52	Right precuneus	529	4.63
42 -39 38	Right inferior parietal lobe		4.12
15 -59 53	Right precuneus		3.67
12 -20 15	Right thalamus		3.63
-21 5 44	Left cingulate gyrus	100	4.56
-27 18 5	Left claustrum	52	4.02
21 18 18	Right caudate	34	4.01
-45 2 41	Left middle frontal gyrus	46	3.98
-45 25 26	Left middle frontal gyrus	34	3.97
-50 19 27	Left middle frontal gyrus		3.85
-6 17 43	Left medial frontal gyrus	28	3.82
33 -3 47	Right middle frontal gyrus	31	3.74
CI patients *vs.* HC			
33 -45 35	Right inferior parietal lobe	44	3.69
-39 -45 38	Left inferior parietal lobe	36	3.61
-45 -42 44	Left inferior parietal lobe		3.56
CI patients *vs.* CP patients			
30 32 1	Right inferior frontal gyrus	22	4.79
-39 -45 38	Left inferior parietal lobe	143	4.63
33 -54 36	Right inferior parietal lobe	214	4.36
33 -65 47	Right superior parietal lobe		4.26
36 -42 35	Right inferior parietal lobe		3.59
-36 42 23	Left middle frontal gyrus	25	3.83
3 -53 -5	Right cerebellum, culmen	41	3.81
-6 20 43	Left cingulate gyrus	35	3.76

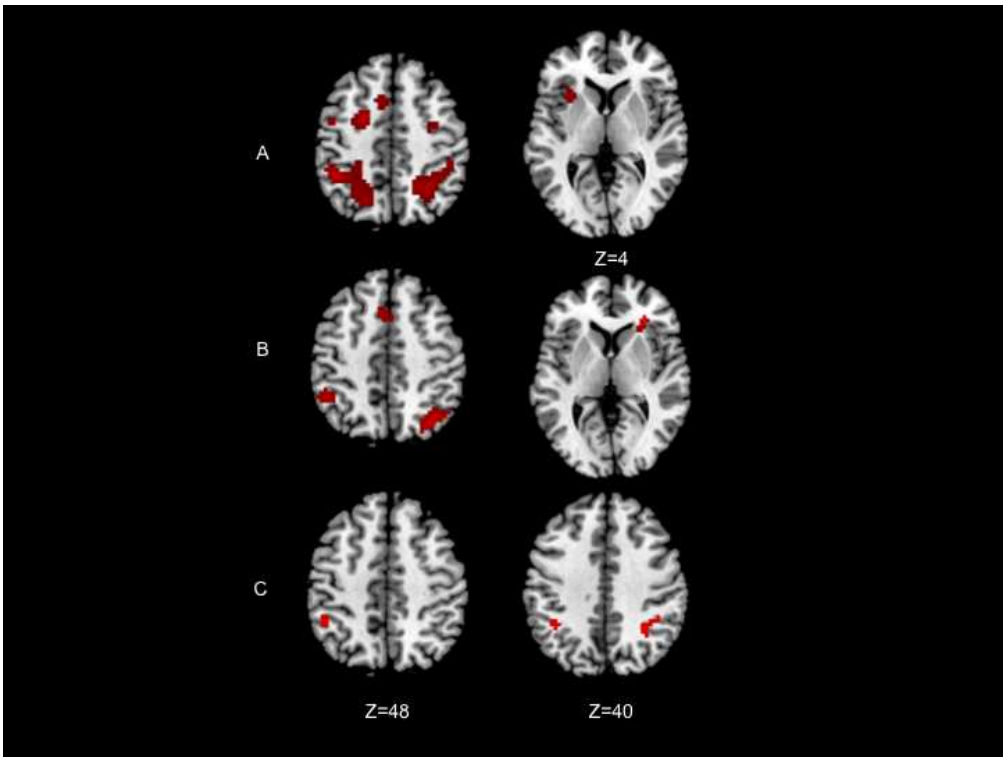
List of abbreviations: HC= Healthy controls; CI=Cognitively impaired; CP=cognitively preserved; MNI=Montreal Neurologic Institute.



A: Cognitive model of working memory used for dynamic causal modeling (DCM) analysis from Au Duong et al. [19]. B: Inter-group differences in effective connectivity in the working memory network during the Paced Auditory Serial Addition Test (PASAT). Dotted black lines show differences between cognitively impaired (CI) patients compared to cognitive preserved (CP) patients. Dashed black lines show differences between CI patients compared to both healthy controls (HC) and CP patients. See text for further results.
254x190mm (72 x 72 DPI)



Areas showing activations during the Paced Auditory Serial Addition Test (PASAT) in healthy controls (A) and patients at presentation with a clinically isolated syndrome suggestive of multiple sclerosis (B). Both groups recruited regions located in the frontal and parietal lobes as well as areas that are part of the working memory network, bilaterally ($p<0.001$ FDR-corrected, $k = 20$). Images are in neurological convention (left is left).
254x190mm (72 x 72 DPI)



A: Areas showing increased activations in patients at presentation with a clinically isolated syndrome (CIS) compared to healthy controls (HC); B: cognitively impaired (CI) patients compared to cognitive preserved (CP) CIS patients; and C: CI patients compared to HC during the Paced Auditory Serial Addition Test (PASAT) ($p<0.05$ FDR-corrected, $k = 20$). Images are in neurological convention (left is left)
254x190mm (72 x 72 DPI)