Magnetic Resonance Study of the Influence of Tissue Damage and Cortical Reorganization on PASAT Performance at the Earliest Stage of Multiple Sclerosis

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Abstract: We sought to determine the influence of tissue damage and the potential impact of cortical reorganization on the performance to the Paced Auditory Serial Addition Test (PASAT) in patients at the earliest stage of multiple sclerosis (MS). Magnetization transfer ratio (MTR) imaging and functional magnetic resonance imaging (fMRI) experiments using PASAT as paradigm were carried out in 18 patients with clinically isolated syndrome suggestive of MS (CISSMS) compared to 18 controls. MTR histogram analyses showed structural abnormalities in patients involving the normal-appearing white matter (NAWM) but also the gray matter (GM). Mean PASAT scores were significantly lower in the group of patients taken as a whole, and were correlated with the mean NAWM MTR value. No correlation was observed between PASAT scores and GM MTR. However, in the subgroup of patients with normal PASAT performance ($n = 9$), fMRI showed larger activations in bilateral Brodmann area 45 (BA45) and right BA44 compared to that in controls ($n = 18$). In these areas with potentially compensatory reorganization, the whole group of patients ($n = 18$) showed significantly greater activation than controls ($n = 18$). Activation in the right BA45 was inversely correlated with the mean NAWM MTR and the peak position of GM MTR histograms of patients. This study indicates that even at the earliest stage of MS, cortical reorganization is present inside the executive system of working memory and could tend to limit the determinant functional impact of NAWM injury on the execution of the PASAT. *Hum Brain Mapp* 24: 216–228, 2005. © 2004 Wiley-Liss, Inc.

Key words: multiple sclerosis; cortical reorganization; fMRI; magnetization transfer; clinically isolated syndrome

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INTRODUCTION

There is increasing evidence that neuroplasticity, a property that allows the central nervous system (CNS) to adapt itself to various brain insults, may limit the clinical expression of tissue damage in patients with relapsing-remitting and both primary- and secondary-progressive multiple sclerosis (MS) [Audoin et al., 2003; Filippi et al., 2002; Lee et al., 2000; Pantano et al., 2002a,b; Reddy et al., 2000a, 2002; Rocca et al., 2002a,b, 2003a,b; Staffen et al., 2002; Yousry et al., 1998]. Adaptive cerebral plasticity of the cortical motor system would be present at the earliest stage of MS in patients with clinically isolated syndrome suggestive of MS (CISSMS) [Pantano et al., 2002a,b; Rocca et al., 2003a]. At that stage, cerebral plasticity may contribute to maintain normal motor performance despite tissue damage [Pantano et al., 2002a; Rocca et al., 2003a]. It seemed relevant to also undertake anatomo-functional studies centered on general cognitive processes like memory, attention, or information processing. Indeed, the frequency of cognitive dysfunction is high in MS, present in nearly half of patients at the early stage of the disease even in subjects with low disability [Amato et al., 1999; Bagert et al., 2002; Dujardin et al., 1998; Rao et al., 1991]. A recent study reported the existence of compensatory cortical activation observed during a complex cognitive task, in patients with clinically definite relapsing-remitting MS [Staffen et al., 2002]. Larger recruitment of brain regions implied in sustained attentional processes was observed in these patients performing the Paced Visual Serial Addition Test (PVSAT) [Staffen et al., 2002], a visual entry version of the Paced Auditory Serial Addition Test (PASAT) [Gronwall, 1977]. We reported recently [Audoin et al., 2003] that cortical reorganization may also exist in high cognitive systems in CISSMS patients in whom a diagnosis of possible MS or MS was made based on McDonald's criteria [McDonald et al., 2001]. Using PASAT as functional magnetic resonance imaging (fMRI) paradigm, the generally accepted reference test for the cognitive evaluation of MS patients [Cutter et al., 1999], we observed in this first study functional changes mainly located in regions involved in executive processing in 10 CISSMS patients compared to 10 controls, whereas task performance recorded inside the MR scanner was not significantly different between the two groups [Audoin et al., 2003]. Cognitive processes involved in the performance of PVSAT are probably slightly different from those recruited during a conventional PASAT and may explain, at least in part, differences in location of activated cortical regions observed between the two studies. Indeed, absence of interference between storage of verbal information and vocalization of a result modifies the strategy employed by subjects. In the PVSAT, visual entry of stimuli removes an interference between output and input modalities, leading to higher performances of subjects, as reported by others [Fos et al., 2000]. Differences between our results and those published by Staffen et al. [2002] may also be related to the different groups of patients studied (i.e., clinically definite MS vs. CISSMS).

The goal of the present study was to determine the influence of tissue damage and the potential impact of cortical reorganization on the performance of PASAT at the earliest stage of MS. To address this issue, we carried out an fMRI experiment in a population of 18 CISSMS patients and 18 controls using PASAT as paradigm to study potential correlations between functional cortical changes and tissue abnormalities assessed by magnetization transfer ratio (MTR) imaging, a sensitive and robust method for detecting and quantifying pathologic changes in MS [Filippi and Grossman, 2002].

SUBJECTS AND METHODS

Subjects

We explored a group of 18 patients with CISSMS fulfilling at inclusion at least the dissemination in space criteria according to McDonald (dissemination in space demonstrated by MRI, or positive cerebrospinal fluid (CSF) plus two or more MRI-detected lesions consistent with MS [McDonald et al., 2001]). Fourteen patients had a diagnosis of MS and four had a diagnosis of possible MS according to McDonald's criteria when comparing the first MRI carried out after relapse and conventional MRI carried out at the same time as fMRI exploration (at least 3 months after onset and steroid treatment). The functional status of subjects was evaluated using the expanded disability status scale (EDSS) [Kurtzke, 1983] and the multiple sclerosis functional composite (MSFC) score [Cutter et al., 1999; Fischer et al., 1999] (Table I). The 18 patients were matched with 18 healthy controls for age, gender, and educational level. Comparison of group characteristics are summarized in Table II. The control group was used as reference for the calculation of the MSFC score. All subjects (patients and controls) were right-handed (>70% Oldfield scale) [Oldfield, 1971], native French speakers, and naive people with respect to PASAT. They gave their informed consent for their participation in this experiment that was approved by the local Ethics Committee (Timone Hospital, Marseille, France).

Conventional and Structural MRI

MRI acquisition

Images were acquired using a 1.5-T Magnetom Vision Plus MR imager (Siemens, Erlangen, Germany). The MRI protocol included localizer scout images, transverse fast spin echo (SE) proton density-weighted and T₂-weighted images (echo time [TE]₁/[TE]₂/repetition time [TR] = 15 ms/85 ms/2,600 ms, 44 contiguous slices, thickness = 3 mm, flip angle = 90 degrees, field of view [FOV] = 240mm, matrix = 256²), transverse proton density-weighted spoiled gradient echo sequences (TE/TR = 4.7 ms/500 ms, 44 contiguous slices, thickness = 3 mm, flip angle = 30 degrees, FOV = 240 mm, matrix = 256²) carried out with and without magnetization transfer (MT) saturation (1.5-kHz off water resonance, 500 degrees). A T₁-weighted SE sequence (TE/TR = 10 ms/650 ms, 25 contiguous slices, thickness = 5mm, flip

TABLE I. Clinical characteristics of CISSMS patients

Patient no.	Age (yr)	Clinical syndrome	McDonald's criteria	Months since onset	EDSS	PASAT	MSFC	T ₂ lesion load (cm ³)
1	22	Optic neuritis	MS	7	1	30	-1.758	1.78
2	35	Hemispheric syndrome	MS possible	7	0	37	-1.108	2.36
3	36	Optic neuritis	MS	3	1	37	-1.961	1.48
4	34	Hemispheric syndrome	MS	12	0	34	-1.213	2.36
5	34	Optic neuritis	MS	4	0	30	-1.106	1.78
6	37	Spinal cord syndrome	MS possible	5	2	21	-2.093	1.32
7	27	Optic neuritis	MS	5	2	36	-1.330	1.74
8	25	Optic neuritis	MS	4	0	32	-0.631	2.03
9	25	Brainstem syndrome	MS	6	0	34	-0.377	1.19
10	36	Hemispheric syndrome	MS	6	1	45	-1.082	5.94
11	22	Spinal cord syndrome	MS	3	1	54	0.354	0.58
12	40	Hemispheric syndrome	MS possible	4	0	51	-0.532	3.38
13	38	Optic neuritis	MS	24	1	40	-1.232	2.20
14	19	Spinal cord syndrome	MS	4	1	41	-0.827	1.02
15	25	Hemispheric syndrome	MS	10	0	49	0.224	0.53
16	22	Spinal cord syndrome	MS	6	1	50	0.576	5.06
17	20	Brainstem syndrome	MS	3	0	48	0.083	2.59
18	30	Spinal cord syndrome	MS possible	7	1	45	0.059	1.28

CISSMS, clinically isolated syndrome suggestive of multiple sclerosis (MS); EDSS, expanded disability status scale; PASAT, Paced Auditory Serial Addition Test; MSFC, MS functional composite.

angle = 90 degrees, FOV = 240 mm, matrix = 256²) was also carried out 5 min after injection of gadolinium (0.1 mM/kg).

MTR image processing

MTR maps were calculated on a voxel-by-voxel basis according to the following equation: $MTR(\%) = (M_0 - M_{mt}) / M_0$, where M_0 and M_{mt} were the images obtained without and with MT saturation pulse, respectively. MTR maps were

coregistered onto the corresponding T₂-weighted images. T₂ lesions were contoured using a semiautomated method (interactive thresholding technique written on the interactive data language (IDL) platform; Research System, Inc.). The T₂ lesion mask was then applied onto the coregistered MTR maps to obtain MTR maps of T₂ lesions, and by difference, MTR maps of the normal-appearing brain (NAB). NAB MTR maps were spatially normalized (nNAB MTR) into the Mon-

TABLE II. Differences in demographics and clinical characteristics in patients and controls

Parameter	All patients (n = 18)	Group A (n = 9)	Group B (n = 9)	Controls (n = 18)
Age (yr)	29.5 (7)	27.7 (7.8)	31.2 (5.7)	25.3 (6.3)
Educational level (yr)	13.1 (2.4)	13.2 (2.0)	12.9 (2.3)	14.7 (2.10)
Months since presenting symptom	6.66 (4.94)	7.3 (6.2)	5.8 (2.8)	—
EDSS median (range)	1 (0–2)	1 (0–1)	1 (0–2)	—
MSFC score	-0.78 (0.8) ^a	-0.264 (0.666)	-1.287 (0.577) ^{a,c}	-0.101 (0.60)
PASAT	39.7 (8.9) ^b	45.7 (6.0)	32.1 (5.3) ^{b,d}	48.72 (6.78)
T ₂ lesion load (cm ³)	2.15 (1.4)	2.5 (1.9)	1.8 (0.4)	—
McDonald's criteria (n)				
MS possible	4	2	2	—
MS	14	7	7	—
Clinical syndrome (n)				
Optic neuritis	—	5	1	—
Hemispheric syndrome	—	2	3	—
Spinal cord syndrome	—	1	4	—
Brainstem syndrome	—	1	1	—

Values are means (standard deviation) unless otherwise indicated. Group A, patients with normal Paced Auditory Serial Addition Test (PASAT) performance; Group B, patients with abnormal PASAT performance. EDSS, expanded disability status scale; MSFC score, multiple sclerosis (MS) functional composite score.

^a $P < 0.05$ compared to controls; ^b $P < 0.005$ compared to controls; ^c $P < 0.05$ compared to Group A; ^d $P < 0.005$ compared to Group A; statistical significance with Mann-Whitney U -test (after significance $P < 0.05$, with Kruskal-Wallis test for multiple group comparisons).

trear Neurology Institute (MNI) space using a T_1 anatomic template provided by SPM99 software (Wellcome Institute, London, UK). Segmentation of nNAB MTR maps using signal intensity and prior knowledge [Friston et al., 1999] resulted in three maps representing grey matter (GM), normal-appearing white matter (NAWM) and CSF relative percentages in pixels, respectively. Masks of the two compartments were computed by selecting pixels showing relative composition in GM and WM higher than 50%. These masks were then applied on the nNAB MTR maps to obtain nGM and nNAWM MTR maps.

MTR histogram analysis

Histograms of nGM and nNAWM MTR were divided by the total number of pixels for each subject to account for differences in brain size. Conventional histogram metrics such as mean MTR value, location and normalized pixel count of the most frequent MTR value (peak of the histogram), 25% percentile (MTR25%), 50% percentile (MTR50%) and 75% percentile (MTR75%) were evaluated. Comparison of these parameters between CISSMS patients and controls were assessed using the nonparametric Mann-Whitney *U*-test.

Correlations between clinical data (MSFC, EDSS, PASAT) and MTR histogram metrics were assessed using the Spearman rank correlation test.

fMRI

Stimuli and design

The fMRI protocol consisted of 180 EPI measurements, divided into four steps. First, a rest period (30 blocks) followed by the PASAT (60 blocks), a second rest period (30 blocks) and the final repetition task (60 blocks). The two rest blocks (one before the PASAT task and the other before the control task) were used for conditioning subjects to the MRI scanner noise but were not used directly in the statistical analysis. PASAT task consisted in auditory entry series of 61 single-digit numbers delivered every 3 s (PASAT 3). After delivery of each number (except the first), subjects were asked to overtly vocalize the result of the addition of the two last numbers heard. Performances of subjects were recorded during fMRI by an investigator present in the examination room and were used to compute the MSFC score of each subject. PASAT was contrasted with a control task consisting in repeating overtly 61 single-digit numbers delivered at the same rate (one number every 3 s) to remove activation related to simple repetition task. Subjects were held tightly in the scanner head coil, asked to close their eyes and were warned and aware of the importance of minimizing head motion during fMRI acquisition, especially when vocalizing results or repeating numbers.

Data acquisition

Functional MR images were acquired using the same scanner as for structural MRI using single-shot gradient-

echo echo-planar imaging (EPI) sequence (TE = 54 ms, bandwidth 1,470 Hz/pixel, 17 slices, 5 mm thickness, 0.5 mm distant factor, matrix = 128^2 , FOV = 240^2 mm², pixel size = 1.88² mm²). Acquisition time for a block of 17 slices was 2.25 s. In total, 180 blocks were acquired at a rate of one block every 3 s. The two series of the 61 single-digit numbers (stimulus duration = 500 ms) were delivered using a personal computer running Cool Edit Pro v1.2 software (Syntrillium Software Corporation, Phoenix, AZ) every 3 s during intervals of silence (blank duration = 775 ms). A morphologic 3-D T_1 -weighted magnetization prepared rapid gradient echo sequence (MPRAGE; TE = 4.7 ms, TR = 9.7 ms, flip angle = 12 degrees, 128 partitions, matrix = 256^2 , isotropic voxel = 1.25³ mm³) was also acquired to superimpose statistical maps.

Data analysis

Images were post-processed using SPM99. After realignment, images were normalized, coregistered, and smoothed with a 8-mm Gaussian filter. Statistical group comparisons were carried out using random-effect analysis [Friston et al., 1999]. After obtaining a single image for each subject, parameterizing the effect of interest, within-group analysis (one-sample *t*-test, $P < 0.01$), and between-group analysis (two-sample *t*-test, $P < 0.005$) were carried out. MNI coordinates were transformed in Talairach's coordinates using a nonlinear transformation. Activation clusters were then assigned as Brodmann areas (BAs). The mean regional activation was determined for each subject in BAs surviving between-group random-effect comparisons. Differences between patients and controls were then assessed using a nonparametric Mann-Whitney *U*-test ($P < 0.05$). Regional mean activation of patients was also used to study correlation between cortical activation and MTR histogram metrics (Spearman rank correlation).

RESULTS

Clinical Characteristics

Patients and controls showed no statistical differences related to age, gender, and educational level. MSFC was reduced significantly in patients ($P = 0.04$), according to a decrease in PASAT scores ($P = 0.004$), with nonsignificant differences in the nine-hole peg test ($P = 0.323$) and in the 25-foot walk ($P = 0.0905$). A low median EDSS of 1 (range, 0–2) was observed in patients with a moderate mean T_2 lesion load of 2.15 ± 1.42 cm³.

To minimize the confounding effect of performances in the location of regions potentially involved in compensatory processes, we selected a posteriori a group of patients with normal PASAT score (mean score of controls ± 1.5 SD). Nine patients fulfilled these criteria. No differences in age, gender, educational level, MSFC score, or PASAT score were found between this group of patients (Group A) and controls (Table II). No differences in age, gender, and educational level were observed between patients with abnormal

Figure 1.
MTR histograms in CISSMS patients and controls. **A:** NAWM MTR histogram. **B:** GM MTR histogram.

PASAT performance (Group B) and controls (Table II). No difference were observed for age and educational level between Groups A and B.

Characteristics of Tissue

No significant differences in T_2 lesion load were observed between Group A ($2.5 \pm 1.9 \text{ cm}^3$) and Group B ($1.8 \pm 0.4 \text{ cm}^3$) of patients (Table II). NAWM and GM MTR histograms of patients and controls are displayed in Figure 1.

Statistical comparisons (Mann-Whitney U -test) showed that mean NAWM MTR value, peak position of the NAWM MTR histogram, and the position of MTR25%, MTR50%, and MTR75% of the NAWM MTR histogram were significantly lower in patients compared to that in controls (Table III).

Mean GM MTR value, pixel count, and position of MTR50% and MTR75% were significantly lower in patients

compared to that in controls (Table III). In all patients, mean NAWM MTR and mean GM MTR were correlated ($r = 0.779$, $P = 0.0013$) as well as in all controls ($r = 0.783$, $P = 0.0012$). No abnormalities in NAWM and GM MTR histogram metrics were observed in patients with normal PASAT performance (Group A) compared to that in controls (Table II).

Mean NAWM MTR values and position of MTR75% of patients with abnormal PASAT performance (Group B) were lower than those of controls and Group A patients (Table III). Peak position and position of MTR25% and MTR50% were lower than that in controls (Table III).

Pixel count of GM MTR25% and GM MTR50% measured in Group B patients were significantly lower than those measured in controls and Group A patients. Mean GM MTR values, peak position, peak height, position of MTR50% and MTR75%, and pixel count of MTR75% of

TABLE III. Normal-appearing white matter and gray matter MTR histogram metrics: comparison between patients and controls

	All patients (n = 18)	Group A (n = 9)	Group B (n = 9)	Controls (n = 18)
Normal-appearing white matter				
Mean MTR (%)	46.81 (1.00) ^a	47.37 (0.82)	46.26 (0.86) ^{b,c}	47.69 (0.53)
MTR peak position (%)	46.99 (0.96) ^a	47.42 (0.96)	46.55 (0.76) ^b	47.71 (0.56)
Normalized pixel count MTR peak	0.030 (0.003)	0.031 (0.003)	0.029 (0.003)	0.030 (0.002)
Position MTR25 (%)	45.56 (1.11) ^a	46.16 (0.875)	44.96 (1.03) ^a	46.30 (0.56)
Normalized pixel count MTR25	0.023 (0.003)	0.024 (0.003)	0.022 (0.004)	0.024 (0.002)
Position MTR50 (%)	46.86 (0.96) ^a	47.14 (1.015)	46.58 (0.873) ^a	47.65 (0.53)
Normalized pixel count MTR50	0.029 (0.003)	0.029 (0.003)	0.029 (0.003)	0.029 (0.002)
Position MTR75 (%)	48.13 (0.86) ^a	48.62 (0.80)	47.65 (0.644) ^{b,c}	49.04 (0.51)
Normalized pixel count MTR75	0.025 (0.003)	0.025 (0.003)	0.024 (0.003)	0.023 (0.002)
Gray matter				
Mean MTR (%)	41.11 (1.13) ^a	41.44 (1.036)	40.78 (1.18) ^a	41.81 (0.55)
MTR peak position (%)	43.08 (1.13)	43.60 (1.035)	42.56 (1.01) ^a	43.78 (0.61)
MTR peak height	0.029 (0.004)	0.031 (0.002)	0.027 (0.004) ^a	0.031 (0.003)
Position MTR25 (%)	39.02 (1.41)	39.35 (1.29)	38.69 (1.51)	39.74 (0.63)
Normalized pixel count MTR25	0.017 (0.001)	0.018 (0.001)	0.016 (0.002) ^{a,c}	0.018 (0.001)
Position MTR50 (%)	41.49 (1.12) ^a	41.84 (1.02)	41.14 (1.17) ^a	42.20 (0.54)
Normalized pixel count MTR50	0.025 (0.002) ^a	0.026 (0.001)	0.023 (0.003) ^{a,c}	0.027 (0.002)
Position MTR75 (%)	43.44 (0.90) ^a	43.77 (0.862)	43.10 (0.851) ^a	44.09 (0.50)
Normalized pixel count MTR75	0.028 (0.004)	0.030 (0.002)	0.026 (0.004) ^a	0.030 (0.003)

Height of the histograms at percentiles 25, 50, 75 and maximum is expressed in normalized pixel count that represent the ratio of the number of pixels at a given percentile by the total number of pixels. MTR, magnetization transfer ratio.

^a $P < 0.05$ compared to controls; ^b $P < 0.005$ compared to controls; ^c $P < 0.05$ compared to Group A; statistical significance with Mann-Whitney *U*-test (after significance with Kruskal-Wallis test for multi-group comparisons).

GM histograms were lower in Group B than in controls (Table III).

fMRI

Brain areas with significant activation in healthy controls are summarized in Figure 2 and Table IV. Compared to

controls, the nine patients with normal PASAT performance (Group A) showed significant greater activation in the left lateral prefrontal cortex (BA45, $P = 0.012$) and right lateral prefrontal cortex, namely in right BA44 ($P = 0.0378$) and in right BA45 ($P = 0.0241$; Mann-Whitney *U*-test) (Fig. 3 and Table V).

Figure 2.
Cortical activation during PASAT in healthy controls (n = 18; one-sample *t*-test, $P < 0.01$, $k > 5$.)

TABLE IV. Brain activation sites in healthy controls with contrast (PASAT-REPEAT)

Brain activation sites (Brodmann area)	κ	Talairach coordinates			t
		x	y	z	
Left lateral premotor cortex (BA6)	126	-19	10	63	4.00
		-28	10	60	3.19
		-36	-2	39	3.35
		-49	17	29	3.07
		-36	-2	59	3.08
Supplementary motor area (BA6)	67	-4	16	46	2.88
Left inferior frontal gyrus (BA44)	50	-43	3	22	3.84
Left inferior frontal gyrus (BA45)	15	-60	15	20	3.01
		-59	16	4	2.94
Left inferior frontal gyrus (BA45/46)	268	-35	28	19	2.87
		-56	26	14	2.78
Left inferior frontal gyrus (BA 47)	62	-55	25	-5	3.01
Left inferior parietal lobule (BA40)	55	-40	-32	49	2.66
		-39	-45	41	2.96
Left superior parietal lobule (BA7)	87	-9	-54	72	3.89
		-28	-47	58	2.88
		-28	-54	62	2.82
		-32	-59	51	2.63
Left middle temporal gyrus (BA21)	92	-45	-10	-14	3.35
		-41	-5	-18	2.99
		-66	-14	-17	2.77
Left fusiform gyrus (BA37)	68	-47	-47	-15	4.85
Left cerebellum	1,865	-36	-66	-30	3.36
		-26	-73	-28	3.04
Right anterior cingulate cortex (BA32)	182	13	17	32	3.43
Right anterior cingulate cortex (BA24)	42	1	4	32	3.10
Right inferior frontal gyrus (BA44)	34	47	3	25	2.94
Right inferior frontal gyrus (BA47)	25	57	35	-3	3.14
		55	36	-14	2.94
Right middle frontal gyrus (BA11)	23	46	56	-15	2.88
Right middle frontal gyrus (BA10)	40	44	56	-7	2.76
		38	60	-4	2.64
Right superior temporal gyrus (BA42/22)	12	69	-31	7	3.32
Right superior temporal gyrus (BA42)	38	71	-24	16	3.10
Right superior temporal gyrus (BA22)	18	67	-38	10	2.87
Right middle temporal gyrus (BA21)	32	37	1	-23	2.92
		42	-58	7	2.82
Right inferior temporal gyrus (BA20)	46	54	-36	-17	3.19
		68	-11	-20	3.05
Right fusiform gyrus (BA20)	72	43	-32	-14	3.85
Right fusiform gyrus (BA21)	15	39	-7	-24	3.77
Right lingual gyrus (BA18)	60	5	-87	-2	3.13
Right insula	28	43	5	14	2.94
Right precuneus (BA19/7)	50	15	-74	30	2.90
Right cerebellum	768	34	-66	-29	3.40

Brain activation sites in healthy controls ($n = 18$) with contrast (PASAT-REPEAT). Random- effect analysis, one-sample t -test, $P < 0.01$, $k > 5$). Regions are listed according to location of activation centroid. Coordinates are determined from the Talairach and Tournoux [1988] atlas.

Compared to controls, the nine patients with abnormal PASAT performance (Group B) also showed significant greater activation in right lateral prefrontal cortex, namely in right BA44 ($P = 0.0093$) and in right BA45 ($P = 0.0019$) (Mann-Whitney U -test). No significant differences in mean

activation were observed in these area between patient Groups A and B.

In bilateral BA45 and in right BA44, mean activations of individual regions were statistically higher in the whole group of patients (with normal and abnormal performances,

A

B

Figure 3.

A: Relative increase in cortical activation during PASAT of CISSMS patients ($n = 18$) in comparison to healthy controls ($n = 18$) (two-sample t -test, $P < 0.01$, $k > 5$). **B:** Relative increase in cortical activation during PASAT of CISSMS patients with normal performance (Group A, $n = 9$) in comparison to 18 healthy

controls (two-sample t -test, $P < 0.005$, $k > 5$). Significant differences (confirmed by individual statistics in these surviving regions, $P < 0.05$, Mann-Whitney U -test) were obtained in the left and right lateral prefrontal cortices (right and left BA45, right BA44).

TABLE V. Differences in activation between patients with normal PASAT scores (n = 9) and controls (n = 18) with Contrast (PASAT-REPEAT)

Brain activation sites (Brodmann area)	κ	Talairach coordinates			t
		x	y	z	
Left inferior frontal gyrus (BA45/46) ^a	87	-41	28	17	3.30
Left middle frontal gyrus (BA46)	72	-50	25	25	3.27
Left inferior frontal gyrus (BA45) ^a	34	-52	20	17	2.90
		-52	20	17	2.90
		-51	31	2	2.88
Left medial frontal gyrus (BA9)	23	-3	44	28	2.94
Left superior frontal gyrus (BA10)	64	-39	57	16	3.07
Left medial frontal gyrus (BA8)	21	-2	32	47	3.48
		-4	29	57	3.03
		-28	43	39	3.08
Left precentral gyrus (BA6)	12	-58	-3	32	2.92
Left precentral gyrus (BA4)	8	-30	-27	50	3.04
Right superior frontal gyrus (BA10)	15	7	63	15	3.15
Right inferior frontal gyrus (BA45) ^a	68	58	20	14	3.13
Right inferior frontal gyrus (BA45/46) ^a	18	56	28	13	3.11
Right inferior frontal gyrus (BA44) ^a	46	62	12	14	2.90
Right lingual gyrus (BA18)	15	18	-74	-2	3.12
Right cerebellum	34	26	-75	-33	3.29
		39	-85	-24	3.16

Random-effect analysis, $P < 0.005$, $k > 5$). Regions are listed according to location of activation centroid. Coordinates are determined from the Talairach and Tournoux [1988] atlas.

^a $P < 0.05$, significance verified with Mann-Whitney U -test on extracted data.

$n = 18$) than in controls ($n = 18$) in right BA45 ($P = 0.0012$), in left BA45 ($P = 0.021$), in right BA44 ($P = 0.005$) (Mann-Whitney U -test). Compared to patients, controls did not show any significantly greater activation.

Correlations

In all patients ($n = 18$), PASAT scores were correlated with the mean MTR value ($r = 0.658$, $P = 0.0068$), the position of the maximum pixel count ($r = 0.528$, $P = 0.030$), the position of MTR25% ($r = 0.508$, $P = 0.037$) and the position of MTR75% ($r = 0.657$, $P = 0.0068$) of the NAWM (Fig. 4A). MSFC was correlated with mean MTR ($r = 0.598$, $P = 0.014$), the location of the maximum pixel count ($r = 0.550$, $P = 0.024$), and the position of MTR75% ($r = 0.614$, $P = 0.0114$) of the NAWM. No correlations were found between EDSS or T_2 lesion load and NAWM histogram metrics.

No correlations ($P > 0.05$) were found between metrics of GM MTR histograms and clinical data (PASAT score, MSFC, EDSS). No correlations were found between GM histogram metrics and T_2 lesion load.

No correlations were found between T_2 lesion load and clinical data (vs. PASAT score, $P = 0.700$; vs. MSFC, $P = 0.830$; vs. EDSS $P = 0.790$; Spearman rank correlation).

In patients ($n = 18$), activation in right BA45 was inversely correlated with the mean NAWM MTR ($r = -0.502$, P

$= 0.038$) (Fig. 4B), the position of the maximum pixel count ($r = -0.480$, $P = 0.049$) of the NAWM MTR histogram. Activation in right BA45 was also inversely correlated with the position of the maximum pixel count ($r = -0.500$, $P = 0.040$) of the GM MTR histogram (Fig. 4C).

DISCUSSION

Tissue Damage Measured by MTR

We find significant NAWM and GM MTR decrease in CISSMS patients compared to that in matched controls. Larger abnormalities were observed in the NAWM compared to that in the GM. We also observed a positive correlation between MTR of NAWM and that of GM ($r = 0.790$, $P = 0.0013$). These results, observed at the earliest phase of MS, bring new insights into the occurrence of a phenomenon described previously in clinically definite multiple sclerosis (CDMS) patients. Indeed, diffused tissue injuries affecting NAWM and GM, evidenced by parametric MRI or histologic studies, have been reported widely in CDMS patients [Allen and McKeown, 1979; Evangelou et al., 2000; Tortorella et al., 2000]. Until now, the stage of the disease at which normal-appearing brain tissue (NABT) abnormalities first appear has not been established. Previous studies have failed to show consistently MTR abnormalities in the NAWM and the GM in

Figure 4.

A: Scatterplot of NAWM MTR vs. PASAT in CISSMS ($n = 18$). **B:** Scatterplot of activations in right BA 45/46 vs. NAWM MTR in CISSMS. **C:** Scatterplot of activations in right BA45/46 vs. GM MTR peak position in CISSMS.

CISSMS patients, possibly in relation to differences in inclusion criteria [Brex et al., 2001; Iannucci et al., 2000; Kaiser et al., 2000; Traboulsee et al., 2002]. In the current study, to include only patients at the earliest stage of MS, we explored a group of 18 patients fulfilling at inclusion, at least, the dissemination in space criteria according to McDonald [McDonald et al., 2001] (dissemination in space demonstrated by MRI, or positive CSF plus two or more MRI-detected lesions consistent with MS).

Correlation studies between histologic data and MTR measurements in CDMS patients have demonstrated that reductions in MTR values were not specific, and may be related to processes as various as edema, marked astrocytic proliferation, perivascular inflammation, demyelination, and axonal loss [Evangelou et al., 2000; van Waesberghe et al., 1999]. However, no direct histologic data are available in CISSMS patients that can identify the major phenomenon occurring at the earliest stage of MS. Few clues have been brought by MR studies carried out in such a population. MR spectroscopy studies, using region-of-interest (ROI) analysis of the NAWM in patients at presentation with CISSMS, reported no N-acetylaspartate (NAA) reductions in white matter (WM) outside T_2 -visible lesions, in accordance with a limited axonal

damage at the earliest stage of MS [Brex et al., 1999; Ranjeva et al., 2003; Tourbah et al., 1999]. A recent study, using MR spectroscopy, MTR, and mean diffusivity in 46 CISSMS patients, described diffuse structural and metabolic changes in corpus callosum that may be interpreted as representing predominantly myelin pathology rather than axonal loss at the earliest stage of MS [Ranjeva et al., 2003]. Another study measuring the concentration of "whole brain" N-acetylaspartate (WBNA), using a nonlocalized proton MR spectroscopy (^1H MRS) sequence, reported significantly reduced WBNA in patients at presentation with CISSMS [Rocca et al., 2003a]. The reduced WBNA concentrations observed could be interpreted as a transient functional axonal impairment secondary to inflammatory changes associated with the recent clinical attack or axonal damage inside and outside the lesions.

Clinical Impact of Tissue Injury

In the current study, clinical impairments of patients were related to cognitive impairments (evaluated with PASAT) but not to upper or lower limb motor impairments (normal scores to nine-hole peg test and 25-foot

walk). Macroscopic tissue damage evaluated by T₂ lesion load and GM damage evaluated with MTR metrics failed to explain the clinical status of patients. PASAT performances were correlated with the extent of NAWM damage. Because subjects have to maintain (one number heard previously) and manipulate verbal information over a short period of time, PASAT is very demanding in regards to verbal working memory processes. One hypothesis may be that integrity of WM is the main factor required to perform correctly a complex working memory task involving several distant brain areas that have to interact synchronously. Many cognitive theories put emphasis on the involvement of the prefrontal cortices and the long-distance connectivity in controlled information processing [Dehaene et al., 1998; Posner and Dehaene, 1994]. Connectivity disturbance in MS patients may be related to WM fiber tracts damage secondary to the presence of demyelinating lesions or diffuse WM damage. Impairment of connectivity efficiency may jeopardize the good execution of PASAT. To some extent, PASAT performances would be an index of connectivity and this could explain at least in part the sensitivity of this test at the earliest stage of the disease. To be carried out correctly PASAT needs efficient long-distance connectivity that may be essential for high cognitive processes, as suggested by normal PASAT performances of patients presenting tissue damage limited to macroscopic lesions (Group A).

Cortical Reorganization

We showed previously that cortical reorganization in high cognitive system could exist as soon as the earliest stage of MS [Audoin et al., 2003]. Cortical functional reorganization in CISSMS patients performing PASAT as fMRI paradigm was located mainly in prefrontal cortices [Audoin et al., 2003]. Various fMRI studies have supported the existence of functional adaptive changes in MS patients during motor tasks [Filippi et al., 2002; Lee et al., 2000; Pantano et al., 2002a,b; Reddy et al., 2000a, 2002; Rocca et al., 2002a,b, 2003a,b; Yousry et al., 1998]. Such cortical reorganization could be compensatory and could limit the clinical expression of tissue damage [Cifelli and Matthews, 2002; Filippi et al., 2002; Lee et al., 2000; Pantano et al., 2002b; Reddy et al., 2000a,b; Rocca et al., 2002a,b, 2003a,b; Yousry et al., 1998].

The analysis carried out in this study aimed to compare fMRI results in controls (*n* = 18) to fMRI results obtained in CISSMS patients with a good PASAT performance (Group A, *n* = 9). This approach was implemented to separate real cortical reorganization from variations in brain activation related to differences in task performance. This has allowed to define cortical regions susceptible to play a compensatory role.

Variations in cortical responses between patients with normal performance (Group A) and controls during PASAT were located in bilateral prefrontal cortices. These differences were both quantitative (higher activation in patients compared to that in controls in left BA45 and right BA44)

and topographic (recruitment of right BA45). Inside these regions, the whole group of patients showed also significantly greater activation than did controls. Various studies in healthy subjects have suggested that working memory performances are related strictly to activation inside the lateral prefrontal cortices that mediate executive processes [D'Esposito et al., 1995; Duncan and Owen, 2000; Osaka et al., 2003, 2004; Smith and Jonides, 1999]. Increased activation in lateral prefrontal cortices is required during monitoring and manipulation of information [Owen et al., 1998; Smith and Jonides, 1999]. Large activation during PASAT in the lateral prefrontal cortices may be related to the greater recruitment of the executing systems operating on the content of working memory in patients. Although this study has not directly demonstrated the true capacity of cortical reorganization to limit cognitive impairment (no direct relationship between increased activation and PASAT scores), striking is the fact that activation observed in the reorganized areas belonging to the executive system of working memory are inversely correlated with the extent of diffuse tissue injury. This inverse correlation could potentially reflect that cortical reorganization tends to limit to some extent the effect of diffuse NAWM injury on the execution of the PASAT.

CONCLUSIONS

This study indicates that diffuse NAWM damage has a determinant functional impact on the execution of PASAT at the earliest stage of MS. In addition, cortical reorganization is present inside lateral prefrontal cortices and is inversely correlated with the extent of diffuse tissue damage. This inverse correlation could potentially reflect the fact that cortical reorganization tends to limit to some extent the effect of diffuse NAWM injury on the execution of PASAT.

To conclude, the combination of structural and functional data obtained by MTR and fMRI may provide useful quantitative parameters to assess better and understand the clinical status of MS patients, especially at the earliest stage of the disease.

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