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White matter damage impairs access to consciousness in multiple sclerosis

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ABSTRACT

Global neuronal workspace theory predicts that damage to long-distance white matter (WM) tracts should impair access to consciousness during the perception of brief stimuli. To address this issue, we studied visual backward masking in 18 patients at the very first clinical stage of multiple sclerosis (MS), a neurological disease characterized by extensive WM damage, and in 18 matched healthy subjects. In our masking paradigm, the visibility of a digit stimulus increases non-linearly as a function of the interval duration between this target and a subsequent mask. In order to characterize quantitatively, for each subject, the transition between non-conscious and conscious perception of the stimulus, we used non-linear regression to fit a sigmoid curve to objective performance and subjective visibility reports as a function of target-mask delay. The delay corresponding to the inflexion point of the sigmoid, where visibility suddenly increases, was termed the "non-linear transition threshold" and used as a summary measure of masking efficiency. Objective and subjective non-linear transition thresholds were highly correlated across subjects in both groups, and were higher in patients compared to controls. In patients, variations in the non-linear transition threshold were inversely correlated to the Magnetization transfer ratio (MTR) values inside the right dorsolateral prefrontal WM, the right occipito-frontal fasciculus and the left cerebellum. This study provides clinical evidence of a relationship between impairments of conscious access and integrity of large WM bundles, particularly involving prefrontal cortex, as predicted by global neuronal workspace theory.

Introduction

Understanding neural mechanisms underlying conscious perception remains an unsolved question in cognitive neuroscience. Whether conscious perception is an early phenomenon localized in posterior occipital and temporal brain areas or whether it requires a larger network involving anterior frontal and parietal areas is still controversial. In fact, some theoretical models support an essential role of distributed long-distance brain networks in visual awareness (Dehaene et al., 2006; Dehaene and Naccache, 2001; Dehaene et al., 2003; Di Lollo et al., 2000). These models have been corroborated by functional neuroimaging studies showing that access of sensory stimuli to conscious report correlated with the activation of higher associative cortices, particularly parietal, prefrontal and anterior cingulate areas (Beck et al., 2001; Dehaene et al., 2001; Del Cul et al., 2007; Feinstein et al., 2004; Gross et al., 2004; Haynes et al., 2005; Kranczioch et al., 2007; Lumer and Rees, 1999; Marois et al., 2004; Sergent et al., 2005). In particular, backward masking paradigm, in which the visibility of a briefly flashed stimulus is reduced when followed after a short delay by a second stimulus called the mask, has been used to differentiate conscious from non-conscious processing of visual stimuli. Dehaene et al. (2001) showed in an fMRI study using a masking paradigm that the difference between masked and unmasked words was the presence of increased activity at distant parietal, prefrontal and cingulate sites, only when words were visible. Using a metacontrast backward masking task, Haynes et al. (2005) showed that activity in higher visually responsive areas (fusiform gyrus, V5/MT, and regions of parietal and prefrontal cortex) showed a better correlation with perception than did activity in stimulus-driven regions of early visual cortex. In a recent study using high-density event-related potentials, Del Cul et al. (2007) found that masked digits elicited early activity in posterior occipito-temporal cortices whether they were subliminal or not. However, when they crossed the threshold for conscious access, they were accompanied by late ex-

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tensive activation in anterior and inferior frontal cortex as well as other focal distant parietal, temporal and occipital sites (Del Cul et al., 2007).

According to global neuronal workspace theory (Dehaene et al., 1998; Dehaene et al., 2003), conscious access of visual stimuli rests upon the extension of the brain activation from perceptual areas to higher associative cortices (prefrontal, cingulate and parietal regions) interconnected by long-distance connections and forming a reverberating neuronal assembly. Thus, according to this theory, the integrity of the white matter (WM) may be one main prerequisite for access to consciousness because of the long-distance connections needed to integrate the information from perception to higher cognitive processing.

Here we used a visual backward masking paradigm, adapted from metacontrast masking (Breitmeyer and Ogmen, 2000), which has been found to provide a reliable and highly reproducible experimental measure of the efficiency of the transition between non-conscious and conscious perception. The perception of masked stimuli was evaluated both objectively (objective performance in comparing the digit to 5) and subjectively (introspective ratings of prime visibility on a continuous scale). For both objective performance and subjective reports, performance varies as a sigmoidal function of the target-mask delay, thus showing that conscious perception suddenly increases at delays of around 50 ms (Del Cul et al., 2006; Reuter et al., 2007). Accordingly, we defined the “non-linear transition threshold” as the value of the target-mask delay at which the sigmoidal curve reaches its inflexion point (the relation of this measure to other classical measures of “threshold” is studied in the discussion). According to the “conscious neuronal workspace” theory, the efficiency of conscious access should be affected by WM injury. In order to test this hypothesis, we applied this methodology on patients suffering from multiple sclerosis (MS), an autoimmune disease of the central nervous system characterized by diffuse WM injury affecting long-distance connectivity (Au Duong et al., 2005a; Au Duong et al., 2005b; Cader et al., 2006).

Recently, we evidenced that patients with relapsing remitting MS have an impaired access to consciousness compared to healthy controls whereas non-conscious processing, as measured by priming, was preserved (Reuter et al., 2007). These findings suggested that conscious access to masked stimuli depends on the integrity of large-scale cortical integrative processes, which involve long-distance WM projections. However, no direct evidence of such a relationship between impaired access to consciousness and large bundles WM damage has been provided yet. One way to evaluate the extent of damage in tissue matrix is to determine the reduction in magnetization transfer ratio (MTR) values, reflecting the decrease in the exchange between the bounded pool of protons (macromolecules) and the free pool of protons (free water). MTR decrease can be assigned to processes as various as edema, marked astrocytic proliferation, perivascular inflammation, demyelination and axonal loss (Evangelou et al., 2000; van Waesberghe et al., 1999), but in MS, MTR has been found to correlate more with the extent of demyelination inside the WM (Chen et al., 2007). Moreover, statistical mapping applied to MTR data has recently been reported as a sensitive tool to evidence subtle local tissue damage in different phenotypes of MS in grey matter as well as in WM (Audoin et al., 2007a; Audoin et al., 2006; Audoin et al., 2004; Ranjeva et al., 2005).

The first aim of the present study was to demonstrate that impaired access to consciousness assessed using a validated paradigm (Del Cul et al., 2006; Reuter et al., 2007) is present in patients with MS as soon as the very first clinical stage of the disease where tissue injury is mainly characterized by WM damage. Secondly, we aimed at evidencing, as predicted by the “conscious neuronal workspace” theory, the direct relationship between impairment of access to consciousness and extent of WM damage assessed by statistical mapping analysis applied to brain MTR imaging.

Materials and methods

Subjects

A group of 18 patients with clinically isolated syndrome (CIS) (a totally new population of patients relative to previous studies reported (Audoin et al., 2004; Ranjeva et al., 2005; Reuter et al., 2007) was recruited between January 2007 and June 2007 at the Department of Neurology (Timone University Hospital of Marseille) according to the following criteria: (1) aged between 18 and 45 years; (2) occurrence of a first presumed inflammatory demyelinating event in the central nervous system involving, the spinal cord, the cerebral hemisphere or the brainstem; (3) no previous history of neurological symptoms suggestive of demyelination; (4) exclusion of alternative diagnoses (lupus erythematosus, anti-phospholipid antibody syndrome, Behcet disease, sarcoidosis, Lyme disease, cerebral arteritis, brain lymphoma, etc.); (5) presence of oligoclonal bands on the cerebrospinal fluid (CSF) analysis; and (6) presence on initial MRI, performed before inclusion, of two or more lesions located in the brain or spinal cord.

A control group of 18 age-, sex- and educational level-matched healthy subjects was also included during the same period. All were right-handed and native French speakers.

Exclusion criteria included alcohol or other drug abuse, optic neuritis history, impaired visual acuity and the existence of scotoma on the visual field. None of the patients had experienced a relapse or received treatment with steroids in the preceding three months. Disability was assessed with the Kurtzke Expanded Disability Status Scale (EDSS) (Kurtzke, 1983). All participants gave their informed consent for their participation in this study, which was approved by the local Ethics Committee (Timone Hospital, Marseille, France).

Neuropsychological testing

Neuropsychological assessment was performed using the Brief Repeatable Battery (BRB) (Boringa et al., 2001).

Visual backward masking task

We performed a visual backward masking paradigm used in several previous studies of normal subjects as well as schizophrenic and MS patients (Del Cul et al., 2007; Del Cul et al., 2006; Reuter et al., 2007). The test was delivered through a PC laptop using the E-Prime software (Psychology Software Tools, Inc). Stimuli presentation began with a central cross fixation. Then, a first number (colour: black, Arabic number, font: courier new, size: 36, back-colour: white) that we will call the “prime” appeared. It was followed, after a variable delay, by a masking shape surrounding the prime position without spatial overlap. This mask contained in its structure a second number, the “target” (colour: black, Arabic number, font: courier new, size: 36, back-colour: white), and 3 letters (M, E, E) (colour; black, font: courier new, size: 36, back-colour: white) (see Fig. 1). The prime was presented for 16 ms and the target for 250 ms. The prime was positioned randomly in one of the four positions at the square tops (1.4° visual angle) relative to the central fixation cross (upper left, upper right, bottom left, bottom right). Visual angle of the target was 1° relative to the central fixation cross. We varied the interval between the prime and the subsequent mask, thus allowing us to progressively unmask the stimulus digit. The delay between the onset of the prime and the onset of the mask could take one out of eight values (0, 16, 33, 50, 66, 83, 100 and 150 ms), referred as the stimulus onset asynchrony (SOA). For the SOA value of 0 ms, the prime and the mask had the same onset, but the target persisted after the prime had disappeared. The stimulus set consisted of 16 pairs of prime and target numbers, consisting in all pairs of the numbers 1, 4, 6 and 9 written in Arabic format. As a consequence, the following factors could be analyzed: response

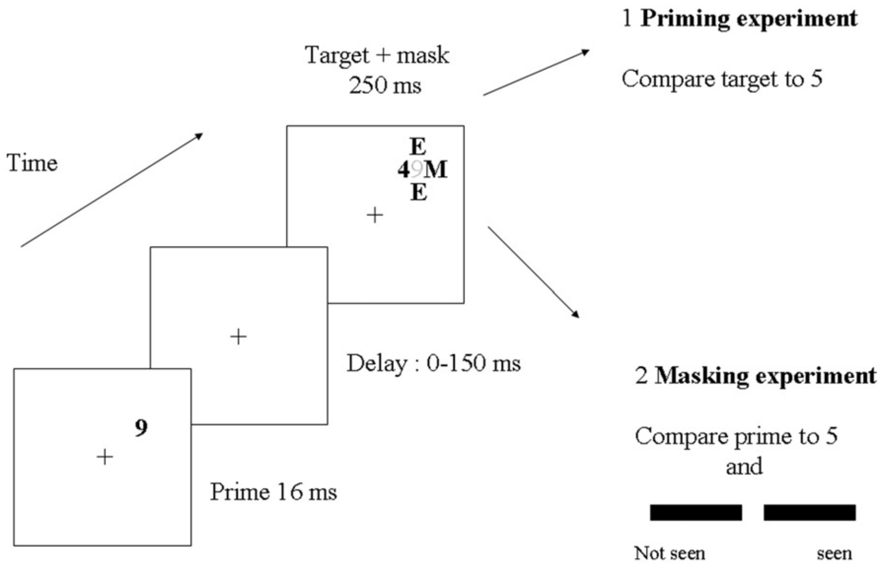

Fig. 1. Experiment design. The prime was presented for 16 ms at one of four positions (1.4° above or below and 1.4° right or left to the fixation cross). The mask (duration of presentation 250 ms) was composed of three letters (M, M, and E) and the target number (1° from the fixation cross). Those four symbols surrounded the prime number without touching it. In the first experiment, referred to as the "priming experiment", subjects were asked to compare each target number with 5, pressing the right-hand key as fast as possible for numbers larger than 5 and the left-hand key for numbers smaller than 5. The second experiment aimed at measuring objective performance and subjective visibility for each delay. We measured objective performance by examining subjects' ability to perform the number comparison task on the prime. Subjects were asked to compare the prime with 5, pressing the right-hand key for numbers larger than 5 and the left-hand key for numbers smaller than 5. We also measured subjective visibility by collecting introspective ratings of prime visibility on a subjective continuous scale.

congruity (whether or not the prime and target fell on the same side of 5), and repetition (within the congruent trials, whether or not the prime and target were the same number). Then, trials could be divided in 3 conditions defined by the prime–target relation: congruent repeated, congruent non-repeated and incongruent.

The experiment began with an explanation of the tasks and a visualization of examples. Two experiments were then performed in a fixed order.

Priming experiment

In the first experiment referred to as the "priming experiment", subjects were asked to compare each target number with 5, pressing the right-hand key as fast as possible for numbers larger than 5 and the left-hand key for numbers smaller than 5. This experiment consisted in 320 experimental trials (8 blocks of 40 trials, one block for each delay). The different delays were blocked in order to facilitate the subject's task; it was felt that, if the delays had been mixed, it would have been too difficult for patients to avoid responding to the prime on conscious trials. Blocking helped them to learn to focus on the target and neglect the prime, regardless of its visibility.

Masking experiment

The experiment consisted in 20 trials for each delay, for a total of 180 trials presented in random order. On each trial, subjects performed an objective and a subjective task. We measured objective performance (% correct) for each SOA by examining subjects' ability to perform the number comparison task on the prime. In this task, subjects were asked to compare the prime with 5, pressing the right-hand key for numbers larger than 5 and the left-hand key for numbers smaller than 5. We also collected subjective ratings of prime visibility. Subjects were asked to move a cursor on a continuous scale from "seen" to "not seen" (Del Cul et al., 2006; Sergent and Dehaene, 2004). This scale was not graduated but was divided by the computer into twenty-one positions (0 to 20).

Both objective and subjective measures were aimed at measuring the delay at which a sudden transition occurs, putatively reflecting the difference between non-conscious and conscious perception. To obtain a summary measure characterizing, for each subject, the transition period between non-conscious and conscious perception of the prime, we used non-linear regression to fit either the objective performance or the subjective visibility curves as a function of SOA with a sigmoid defined as $f(x) = \alpha_1 + \frac{\alpha_2}{1+e^{-\alpha_3(x-\alpha_4)}}$ where the α_i are free parameters. The delay corresponding to the inflexion point of the sigmoid was used as a measure that best characterizes when the transition between non-conscious and conscious perception occurs. This measure, which we term the "non-linear transition threshold", reflects the target-mask delay where the variation in performance or visibility is the highest. Determining such a delay in a region of the curve with large non-linear variation minimizes the errors and allows a better sensitivity to compare the two populations. Our data gives rises to separate objective and subjective evaluations of this non-linear transition threshold, but as we shall see, the two measures are tightly correlated across subjects, confirming our hypothesis that the inflection point marks a major transition point in perception (see also Del Cul et al., 2007, for brain-imaging evidence).

MRI exploration

Patients were imaged with a 1.5-T commercially available unit (Magnetom Vision Plus; Siemens, Erlangen, Germany). The MR imaging protocol included localizer scout imaging, transverse fast spin-echo proton density-weighted and T_2-weighted sequences (2600/15/85 [TR/TE1/TE2], 44 contiguous sections, 3-mm section thickness, 90° flip angle, 240-mm FOV, 256×256 matrix), and transverse proton density-weighted spoiled gradient-echo sequences (500/4.7 [TR/TE], 44 contiguous sections, 3-mm section thickness, 30° flip angle, 240-mm FOV, 256×256 matrix) performed without and with MT saturation (1.5-kHz off-water resonance, 500°). A T1-weighted spin-echo sequence (650/10 [TR/TE], 44 contiguous sections, 3-mm section thickness, 90° flip angle, 240-mm FOV, 256×256 matrix) was also performed 5 min after injection of a gadolinium-based contrast agent (0.1 mmol/L/kg Dotarem, Guerbet, Roissy, France).

MTR image processing (Fig. 2)

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 (Fig. 2). M_{mt} images were coregistered onto the corresponding T_2-weighted images and spatial transformations were applied to the corresponding MTR images. MS lesions were contoured onto the T_2-weighted images using a semi automated method (interactive thresholding technique written on the interactive data language (IDL) platform; Research System, Inc.). The T_2-weighted images were then normalized (16 non-linear registration 7×6×7 basis functions) into the Montreal Neurology Institute (MNI) space using the T_2 anatomical template provided by SPM2 software (Wellcome Institute, London, UK). This transformation applied to both coregistered MTR maps and lesion maps to obtain the normalized MTR maps and the normalized lesion maps. The normalized normal appearing brain tissue maps (NABT=normalized T_2-weighted images−normalized lesion maps) were segmented using the SPM2 software (Friston et al., 1999). A binary mask of the normalized normal appearing white matter (NAWM) mean was obtained by selecting pixels composed by more than 75% in WM from the segmented compartment. The binary mask of normalized lesions was combined to the binary mask of the normalized NAWM. This resulting mask of normalized NAWM and normalized lesions was applied to the normalized MTR map in order to obtain the individual normalized WM MTR map. This map was smoothed with a Gaussian filter (FWHM 12 mm) before statistical assessment.

Statistical mapping analysis

Correlations between local MTR values inside WM and the objective non-linear transition threshold were assessed using statistical mapping analysis (SPM2) (regression with age as confounding covariate, $p<0.005$, $k=20$, uncorrected for multiple comparisons). Significant clusters were then re-tested using a Spearman rank test ($p<0.05$, corrected for multiple comparisons).

Results

Demographics and clinical characteristics of subjects

In patients, mean age was 31.2 years (SD=8.8), mean educational level was 13.4 years (SD=2.3), mean disease duration was 7.6 months (SD=3.8) and median EDSS was 1 (0–1.5). The mean T_2 lesion load was 2.45 mm^3 (SD=2.18) in patients. In controls, mean age was 27 years (SD=9) and mean educational level was 14.3 (SD=2.8). No significant difference was observed between patients and controls in term of age and educational level (Mann Whitney *U*-test). Half of patients (9/18) had at least one post-gadolinium enhancing lesion.

Brief neuropsychological assessment

A significant between-group difference was found in Word List Generation from the BRB (23+21 in patients; 27+8.6 in controls; $p=0.01$) (see Table 1).

Subliminal and conscious priming effects

In the priming experiment, we measured the ability of the subliminal number prime to influence the processing of the subsequent conscious target. We selected the delays where the prime was always processed non-consciously for both groups (SOA 16 ms and SOA 33 ms) and those where the prime was always processed consciously (SOA 100 ms and SOA 150 ms). We then performed an analysis of variance (ANOVA) on mean reaction time (RT) with factors of group, prime–target relation (congruent repeated, congruent non-repeated, and incongruent) and type of process (conscious and non-conscious).

The RT analysis revealed that there was no significant difference between patients and controls both for conscious processing (669.35 ms versus 636.38 ms, $p=0.18$) and for non-conscious processing (586.38 ms versus 564 ms, $p=0.13$). The priming effect on RT was significant in patients both for conscious processing ($p=0.001$) and for non-conscious processing ($p=0.05$). The priming effect was

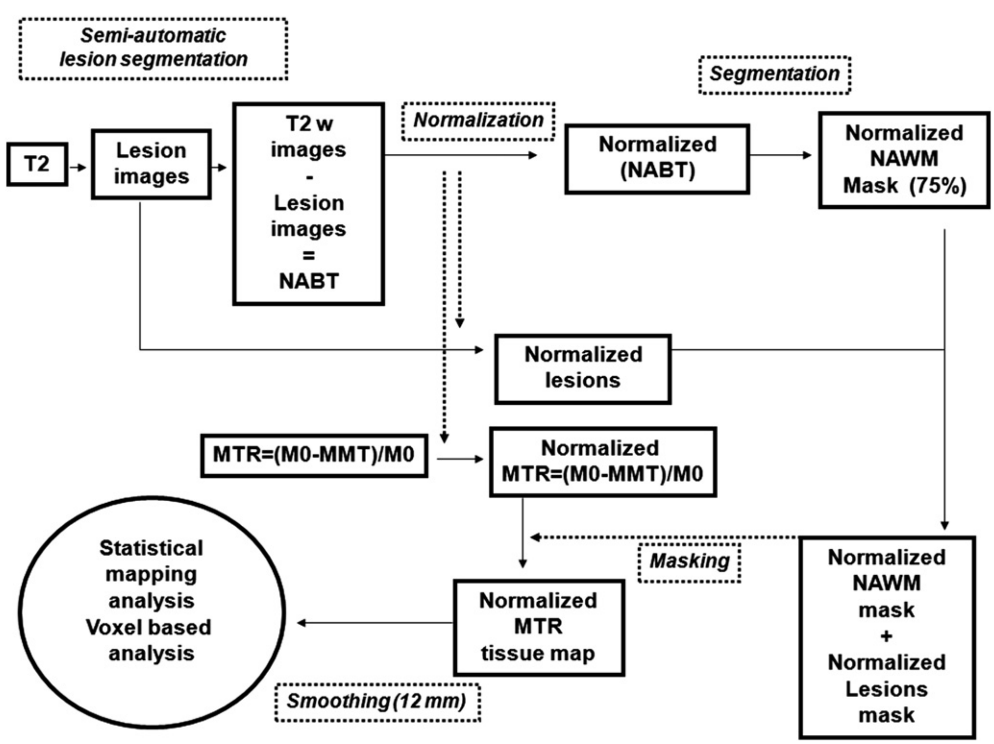

Fig. 2. Processing chain for magnetization transfer ratio images.

Table 1
Performance to the brief repeatable battery

	CIS patients	Controls	*p* value
SRT LTS	57 (10)	61 (7)	0.268
SRT CLTR	54 (12)	56 (8)	0.808
SRT DR	11 (1)	11 (0.5)	>0.999
SPART	20 (5)	20 (4)	0.653
SPART DR	7 (2)	7 (2)	0.958
SDMT	54 (9)	56 (7)	0.416
PASAT 3	44 (8)	48 (7)	0.116
WLG	**19** (7)*	27 (9)	0.01

$p<0.05$, Mann Whitney *U*-test.
All scores are expressed as means (SD). SRT, selective reminding test; LTS, long term storage; CLTR, consistent long term retrieval; DR, delayed recall; SPART, spatial reminding test; SDMT, symbol digit modalities test; PASAT, paced auditory serial addition test; WLG, word list generation.

also significant in controls both for conscious processing ($p=0.001$) and for non-conscious processing ($p=0.01$). There was no group*-priming effect interaction neither for conscious processing ($p=0.9$) nor for non-conscious processing ($p=0.7$).

Characterisation of the transition between non-conscious and conscious perception

To measure the efficiency of the transition from non-conscious to conscious perception, we evaluated the target-mask delay corresponding to the inflexion point of the visibility rate curve, i.e. the time where the variation in performance and visibility is the highest (parameter α_4, see Materials and methods). This measure, termed the "non-linear transition threshold", was obtained from both objective performances and subjective visibility performances (Fig. 3). The mean objective non-linear transition threshold was 57 ms (SD=10)

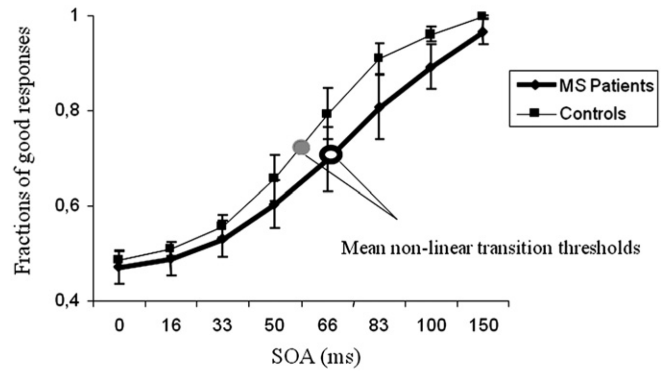

Fig. 4. Objective performance in patients and controls. Fraction of good responses in prime comparison to 5 as a function of SOA and sigmoidal fits (continuous line) are represented separately for patients and controls. The non-linear transition threshold corresponds to the inflexion point of the sigmoid. (The present sigmoidal curves were obtained by averaging the individual subjects' fitting curves for each SOA). Error bars represent the standard error.

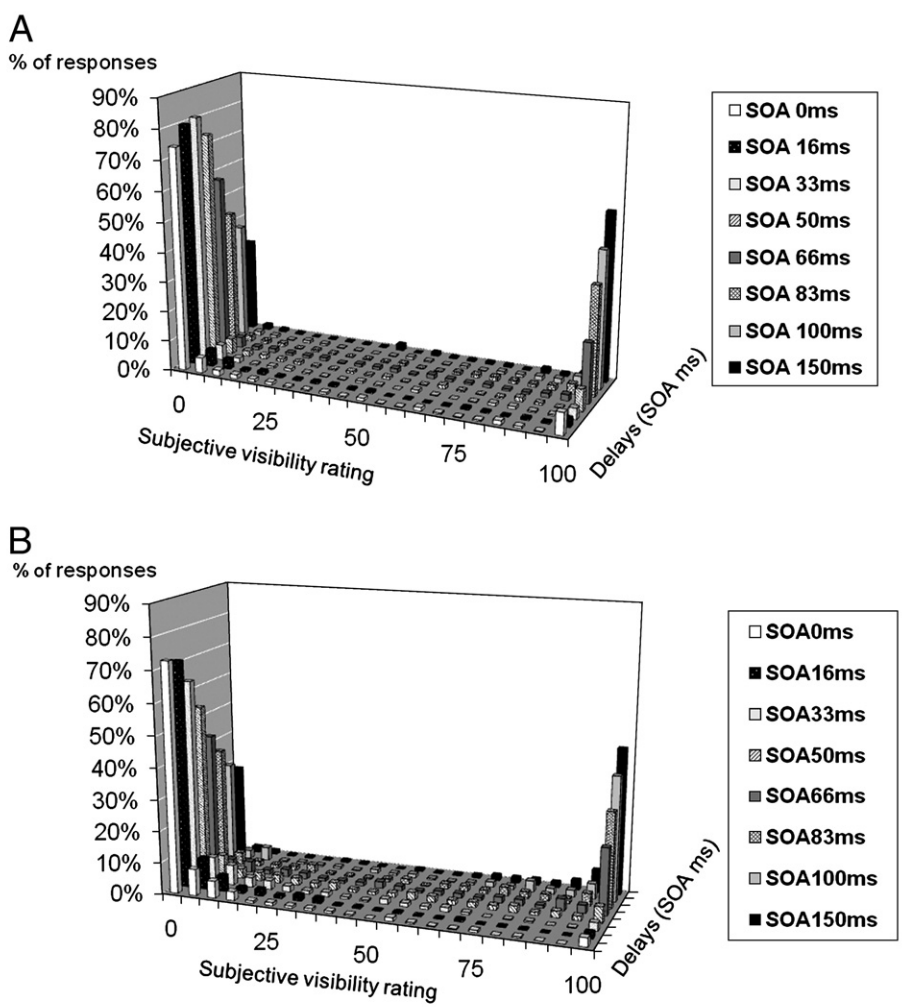

Fig. 3. Distribution of subjective visibility ratings in controls (A) and in patients (B). We observed a bimodal distribution of scores in controls (A) and in patients (B), with a first set of responses close to maximal visibility (scale score>16) and a second set of responses peaking at zero visibility (scale score<4) suggesting that conscious perception is driven by an all or none process.

Table 2
Location of significant inverse correlations between non-linear transition threshold values and local WM MTR (regression with age as confounding covariate $p<0.005$, $k=20$; SPM2)

Location	Cluster extent	Corrected-Rho (Spearman rank)	Corrected *P* (Spearman rank)	Talairach coordinates		
				X	*Y*	*Z*
Right medial anterior prefrontal WM	69	−0.648	0.061	−14	54	−9
				−20	56	−4
				−14	56	3
Left superior prefrontal WM	18	−0.661	0.051	14	57	17
Right dorsolateral prefrontal WM	34	−0.771	0.027*	−38	25	28
				−38	32	24
Right occipito-frontal fasciculus	395	−0.687	0.037*	−22	−14	30
				−22	11	20
				−22	3	24
	145	−0.635	0.070	−22	27	2
Left occipito-frontal fasciculus	18	−0.639	0.067	24	−10	28
Left inferior longitudinal fasciculus	139	−0.660	0.052	40	−45	−1
				34	−62	3
Brainstem	65	−0.635	0.070	−10	−20	−21
Left cerebellum	15	−0.671	0.045*	16	−54	−31

* Statistical values surviving multiple comparison correction (Spearman rank corrected $p<0.05$; 8 comparisons).

for controls (mean fraction of good response at threshold = 0.724 (SD = 0.036)) and 69 ms (SD = 16) for patients (mean fraction of good response at threshold = 0.716 (SD = 0.047). This value was significantly higher for patients than for controls ($p=0.01$, ANOVA with age and educational level as confounding covariates) (see Fig. 4). The mean value obtained from the subjective visibility curves was 59 ms

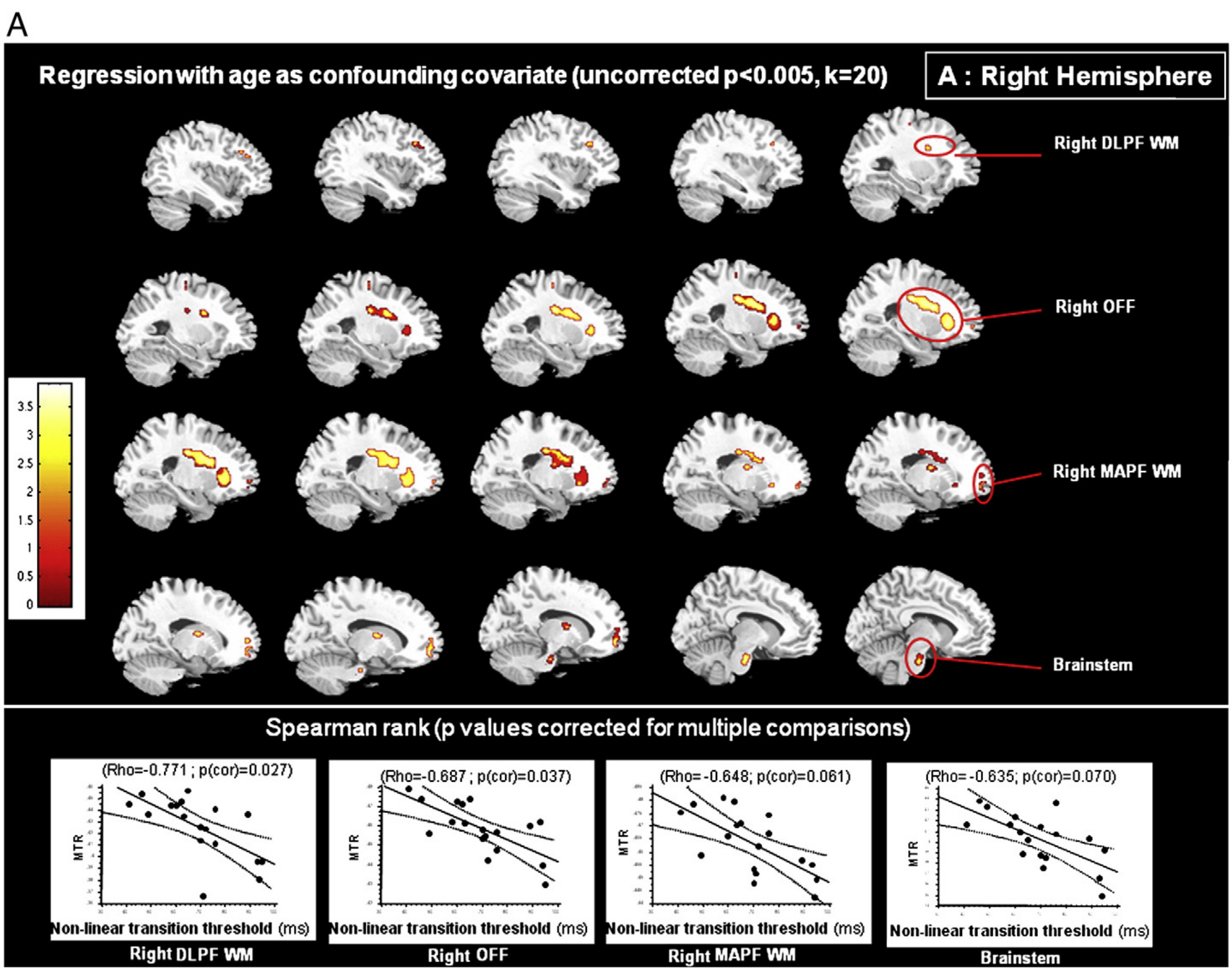

Fig. 5. Statistical maps of the correlations between local MTR values and the non-linear transition threshold in CIS patients. Results from voxel based analysis (regression with age as covariate, uncorrected $p<0.005$, $k=20$) are displayed for the right hemisphere (A) and the left hemisphere (B). Significant clusters were then re-tested using a Spearman rank test ($p<0.05$, corrected for multiple comparisons). DLPF WM: Dorsolateral Prefrontal White Matter; OFF: Occipito-Frontal fascicle; MAPF WM: Medial Anterior Prefrontal white matter; ILF: Inferior Longitudinal fascicle; SPF WM: Superior Prefrontal white matter.

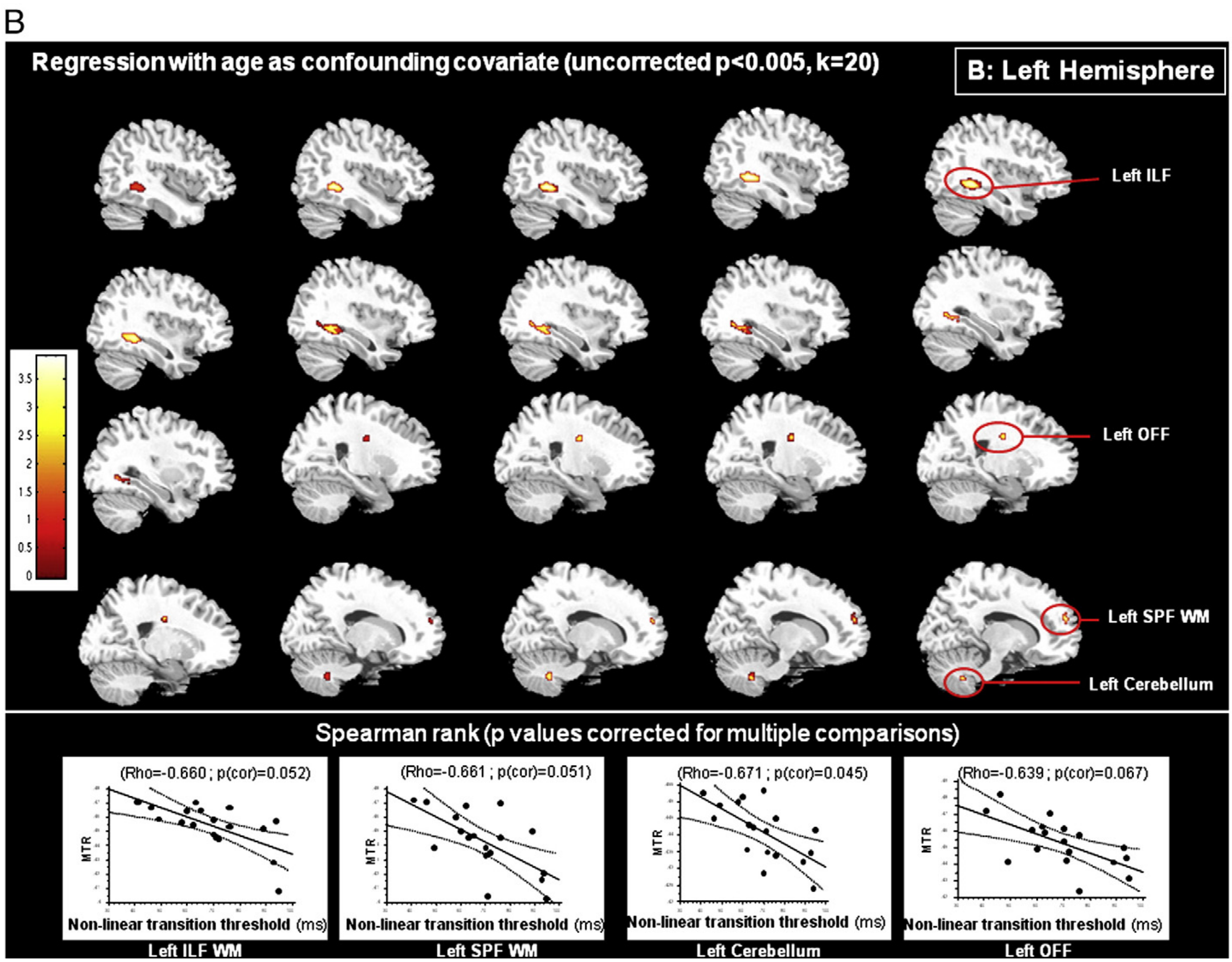

Fig. 5 (*continued*).

(SD = 10) for controls and 73 ms (SD = 13) for patients. Again, this value was significantly higher for patients than for controls (p = 0.0005, ANOVA with age and educational level as confounding covariates). In fact, objective and subjective non-linear transition thresholds were tightly correlated within the controls (corrected Rho = 0.872, p = 0.0003, Spearman rank test) as well as within the patients (corrected Rho = 0.686, p = 0.005, Spearman rank test). In the present populations, no direct correlation was observed between these values (both subjective and objective) and age or educational level.

Relationships between access to consciousness and WM MTR in patients

Data are displayed in Table 2 and Figs. 5A and B. Given the correlation between objective and subjective non-linear transition thresholds, we arbitrarily used the objective values as a parameter for correlation with white matter injury in the patients. We first looked for negative correlations between 'non-linear transition threshold' and MTR values, indicating that an elevated value was associated with a local WM injury. Significant negative correlations were observed in patients inside the right dorsolateral prefrontal WM, the right occipito-frontal fasciculus and in the left cerebellum. We found trends for negative correlations in the right medial and bilateral superior prefrontal WM, in the left occipito-frontal fasciculus, in the left inferior longitudinal fasciculus and in the brainstem. No positive correlations were observed. We also did not find any correlation with T_2 lesion load.

Discussion

The present study suggests that the process of conscious access of masked stimuli is altered at the very first clinical stage of MS, and shows, for the first time, a direct relationship between large-scale WM damage revealed by local MTR decrease and abnormal and delayed transition towards conscious perception during masking.

Measuring a 'non-linear transition threshold' between non-conscious and conscious perception

The present work uses the measurement of the inflexion point of the sigmoidal curve relating either objective performance or subjective visibility to target-mask SOA as a marker of the transition period between non-conscious and conscious perception. This aspect of our research is based on prior results, replicated here, which shows that in some metacontrast masking, substitution masking and attentional blink tasks, participants' reports of seeing the stimulus evolve in a highly non-linear manner with SOA (Del Cul et al., 2007; Del Cul et al., 2006; Reuter et al., 2007; Sergent et al., 2005; Sergent and Dehaene, 2004). Several findings suggest that this non-linearity reflects a major event in the brain's processing of masked stimuli, justifying our attempt to use it as a marker of brain injury. Even when participants are given an opportunity to respond with a continuous rating of visibility, their responses are distributed in a bimodal manner: on any given trial, they either report very high visibility or very reduced visibility. Thus, the smooth sigmoidal curve actually reflects the average of different proportions of seen and not-seen judgments, and the inflexion point characterizes the point of maximal variability in these proportions. Recordings of brain activation at several levels of masking (Del Cul et al., 2007) confirm that a sharp non-linear phase transition underlies the sudden feeling of "seeing" a masked stimulus. Note that the inflexion point is just a convenient, robust measure of this shifted non-linearity. Determining such a delay in a region of the curve with large gradient variations minimizes the measurement error and allows for a better sensitivity when comparing the two populations.

It is important to note that our definition of 'non-linear transition threshold' differs from the classical psychological definition of "objective threshold" and "subjective threshold", Objective threshold is classically defined as the target-mask delay at which performance in some objective task (e.g. target classification) drops down to chance level, and subjective threshold as the delay at which subjects deny detecting the stimulus (Cheesman and Merikle, 1984). The subjective threshold is usually higher than the objective threshold, thus defining a zone of objective "subliminal" performance without subjective reportability. In our prior research (Del Cul et al., 2006), we found such a dissociation at short SOAs, where a non-negligible proportion of subjectively not-seen trials were accompanied by above-chance objective performance. In Cheesman and Merikle's terminology, our results would indicate a very low "objective threshold". The significance of these definitions, however, remains heavily debated (Dehaene and Naccache, 2001; Holender, 1986; Merikle et al., 2001). Several authors have noted that the thresholds are difficult to measure reliably since they depend on the exact instructions, the statistical level of significance chose, and the amount of data collected. Accepting the null hypothesis of a zero d-prime in an objective task can under-estimate conscious access. Conversely, higher-than-chance performance can occur while subjects deny seeing anything, and thus the objective threshold can over-estimate conscious access.

Our study sidesteps many of these difficulties, while focusing on what is, arguably, the most salient finding in the masking literature since over a century: the non-linearity of the curve relating perception to the target-mask delay, indicating that beyond a critical delay, subjects exhibit a sudden ability to extract information and become aware of the masked stimulus. In the present, this non-linearity is also the most evident difference between the controls and the patients: there is a systematic shift of both objective and subjective curves towards higher SOA values in patients, and our measure of 'non-linear transition threshold' adequately captures this phenomenon. Future work should return to the largely orthogonal issue of whether the point of first occurrence of a deviation from chance-level responding also differs between patients and controls (but see Fig. 4 for a clear illustration of the difficulty of assessing this point).

Subliminal priming effect

As reported in relapsing remitting MS (RR-MS) (Reuter et al., 2007), patients with CIS, showed significant subliminal priming effect suggesting normal non-conscious processing. However, we found normal reaction times in CIS patients while RR-MS were slower than controls. The longer RT found in RR-MS patients could be due, at least in part, to motor disability or/and to slower information processing speed.

Impairment of access to consciousness in patients

As previously described, the "conscious neuronal workspace" theory emphasizes the role of long-distance connections necessary for the emergence of a self-amplified reverberant neuronal assembly which connects together distant brain areas, particularly prefrontal, cingulate and parietal cortices, and which is associated with conscious reportability (Dehaene et al., 1998; Dehaene et al., 2003). Diffuse demyelinating processes in patients with MS may disturb the connectivity between distant cortical areas (Au Duong et al., 2005a; Au Duong et al., 2005b; Audoin et al., 2007b), and thus provide a mechanism for impaired conscious access. The load of brain MRI-visible lesions, which is generally low at the early stage of MS, may not account significantly for the degree of impairment of access to consciousness as suggested by the lack of correlation between the T_2 lesion load and the non-linear transition threshold in the present study. This is not surprising considering the results of numerous MRI studies (Allen and McKeown, 1979; Audoin et al., 2005; Audoin et al., 2007b; Cercignani et al., 2001; Cercignani et al., 2000; Evangelou et al., 2000; Tortorella et al., 2000) which demonstrated that pathological processes could be present outside MRI-visible lesions, in the so called 'normal appearing brain tissue' since the very first stage of MS.

The deficit observed in patients is not due to damage to early visual pathways

As discussed in our previous report on relapsing remitting MS (Reuter et al., 2007), impairment of conscious access cannot be related to a dysfunction along the visual pathway including the optic nerve. Because our measures are obtained from the variation in performance as a function of target-mask interval, they should not be affected by an additive optic nerve delay. A second argument against putative low-level visual deficits is the finding of a normal subliminal priming effect in MS patients (Reuter et al., 2007). Behavioral and neuroimaging studies of subliminal priming suggest that masked invisible stimuli are processed extensively, including visual recognition, but also lexical and even semantic levels (Dehaene, 2004; Dehaene et al., 2004; Dehaene et al., 2001; Greenwald et al., 1996; Naccache and Dehaene, 2001). The preservation of non-conscious priming in very early MS patients suggests that the fast feed-forward processing stages supporting these priming effects must be largely intact, including conduction from optic nerve to visual cortex.

The fact that the present study used a visual task to assess non-conscious and conscious processing in MS patients might raise the question of a selection bias with the exclusion of patients with optic neuritis, one of the most common clinical onsets of MS. However, it is noteworthy to state that the first goal of this preliminary study was to demonstrate the important involvement of large WM bundles integrity on access to consciousness. To generalize this approach and allowing a clinical transfer of the method to the whole population of MS patients, it will be crucial to develop paradigms in other sensory modalities, such as auditory perception, for which peripheral injury is far less frequent in early clinical stage of MS.

White matter damage impairs access to consciousness

In the present study, significant negative correlations indicated that the severity of the anomaly in the transition to conscious perception (delayed inflexion point) was related to the impaired MTR values in the right dorsolateral prefrontal WM, the right occipito-frontal fasciculus and in the left cerebellum. We found trends for negative correlations in the right medial and bilateral superior prefrontal WM, in the left occipito-frontal fasciculus, in the left inferior longitudinal fasciculus and in the brainstem. The occipito-frontal fasciculus is a cortical associative fiber tract which extends from the dorsal and medial part of the occipital lobes, the dorsal, medial and inferior parietal lobes to the dorsal and medial part of the prefrontal and premotor regions (Makris et al., 2007). The inverse correlation between the degree of tissue injury of the occipito-frontal fasciculus and the 'non-linear transition threshold' may be related to the strategic anatomical situation of this WM bundle which may be involved in the extension of the brain activation from perceptual occipital areas to associative prefrontal cortices. The inferior longitudinal fasciculus is a WM associative tract connecting the occipital and temporal lobes. Fibers originate from extrastriate visual associative areas of the occipital lobe, sparing the primary visual cortex (Schmahmann and Pandya, 2006). At the occipital horn of the lateral ventricle, the inferior longitudinal fasciculus fibers run laterally to the optic radiation and callosal (tapetal) fibers and fibers terminate in lateral and medial temporal areas. In summary, both the inferior longitudinal fasciculus and the occipito-frontal fasciculus connect visual inputs to higher cortical areas in the occipito-temporal and frontal pathways. In the context of the 'conscious neuronal workspace' theory, these bundles may play a critical role in linking specialized perceptual visual processors to higher-level prefrontal, parietal and cingulate cortices that enter into coherent self-sustained activation patterns during conscious processing. In a recent study (Del Cul et al., 2007), we used high-density EEG to directly monitor the cortical progression of a masked visual stimulus (identical to the present work) as a function of its visibility. We found that invisible stimuli triggered an initial activation in contralateral occipito-temporal cortex and posterior parietal cortices, but that only visible stimuli propagated further into the temporal lobes and especially into inferior prefrontal cortex, leading to a late (>270 ms) surge of global activity distributed bilaterally within prefrontal, posterior parietal and ventral occipito-temporal cortices (Del Cul et al., 2007). Impaired inferior longitudinal fasciculus and occipito-frontal fasciculus fiber tracts would significantly slow this posterior-to-anterior communication and global reverberation, thus providing plausible though still hypothetical neural mechanism relating WM injury to altered access to consciousness.

Interpretation of the correlations found between impaired conscious access and MTR values in the brainstem and the left cerebellum is less clear. However, few studies have shown the involvement of brainstem in attentional processes through the ascendant reticular activating system that could be impaired in patients with CIS (Gadea et al., 2004 6). Concurrently, one functional connectivity experiment has demonstrated significant correlations between low-frequency fluctuations in MR signal of the dentate nucleus and signal fluctuations in cerebellar, thalamic, limbic, striatal, and cortical regions including parietal and frontal sites, with prominent coherence in dorsolateral prefrontal cortex (Allen et al., 2005). This suggests the presence of functional coherence in a large-scale network including cerebellar–parietal and cerebellar–prefrontal functional connections that could be also involved in the consciousness network.

The present study evidences that impaired access to consciousness is present since the very early stage of MS and is related to early injury of long-distance WM bundles. At the theoretical level, it provides the first clinical evidence of a direct relationship between access to consciousness and integrity of large WM bundles, as predicted by the global neuronal workspace theory.

Conflict of interest statement

Any authors declare that they have no conflict of interest relative to this research.

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