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Spectroscopic axonal damage of the right locus coeruleus relates to selective attention impairment in early stage relapsing-remitting multiple sclerosis

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Summary

Lower levels of N-acetylaspartate (NAA), a marker of axonal damage, have been found in the normal-appearing white matter (NAWM) of relapsing-remitting multiple sclerosis (RRMS) patients with low physical disability. However, its relation to the clinical status of these patients remains unclear. We explored the association between NAA levels [normalized to creatine (Cr), NAA/Cr] and a cognitive feature that is not measured by the standard scales that address functional disability [e.g. Expanded Disability Scale Score (EDSS)] in early RRMS. Given that a considerable number of RRMS patients present attentional dysfunction early in the disease and assuming a functional-anatomical oriented guide, it was hypothesized that patients with worse attentional performance would show lower NAWM NAA/Cr values in the locus coeruleus nuclei of the pontine ascendant reticular activating system. Proton magnetic resonance spectroscopy (^{1}H-MRS) examinations with concurrent clinical evaluation were acquired for 19 RRMS patients with a mean evolution time of 24 months (range 10–60) and mild disability (EDSS 0–3.5, median = 1). ^{1}H-MRS was obtained with spectroscopic imaging and measures were taken from the right and left hemipons. Attention was measured by means of the dichotic listening (DL) paradigm to increase the sensitivity of the testing to subtle attentional deficits. A consonant-vowel DL test was measured with and without attentional instructions. For the attentional condition, the test was digitally manipulated to cue automatically to the ear to be attended, thus allowing the obtention of both a linguistic lateralization index (LI) and an index of integrity of attentional shifts (ASI). Attentional impairment was demonstrated in 47.3% of the patients. Pontine NAA/Cr levels accounted for 39% of the ASI variability ($\beta = 0.65$, $P < 0.002$) but did not relate to the LI. Moreover, when NAA/Cr levels were considered separately as left and right hemipons values in a multivariate stepwise linear regression model, the right NAA/Cr ratio alone explained 43% of the ASI variability ($\beta = 0.68$, $P < 0.001$). Since the RRMS patients with greater attentional disturbances exhibited the lowest NAA/Cr levels, it is concluded that NAA provides a specific measure of pathological changes that are also relevant for cognitive functions. The use of both ^{1}H-MRS and DL showed the connection between axonal damage at right locus coeruleus and auditive selective attention dysfunction in early-stage RRMS.

Keywords: relapsing-remitting multiple sclerosis; attention; N-acetylaspartate; proton magnetic resonance spectroscopy; dichotic listening

Abbreviations: ARAS = ascending reticular activating system; ASI = attentional shift index; Cr = creatine; DL = dichotic listening; FL = forced left condition; FR = forced right condition; ^{1}H-MRS = proton magnetic resonance spectroscopy; LE = left ear; LEA = left ear advantage; LI = lateralization index; NAA = N-acetylaspartate; NAWM = normal-appearing white matter; NF = non-forced condition; RE = right ear; REA = right ear advantage; RRMS = relapsing-remitting multiple sclerosis.

Introduction

Multiple sclerosis is a neurological degenerative disorder mainly affecting the white matter, which causes demyelinating plaques, axonal damage and, eventually, neuronal loss (Steinman *et al*., 2002). Over the last few years, a wider interest has been shown in the axonal pathology process in addition to demyelination, with evidence accumulated for the existence of a diffuse axonal injury involvement beyond the multiple sclerosis plaque (Matthews and Arnold, 2001). These data come mainly from proton magnetic resonance spectroscopy (^{1}H-MRS), a non-invasive technique that provides information of microscopic biochemical changes that cannot be detected by a conventional MRI examination (Filippi, 2001). ^{1}H-MRS assessment of N-acetylaspartate (NAA), a metabolite almost exclusively found in neurons (Moffet *et al*., 1991; Simmons *et al*., 1991), has recently been validated in animal models as an axon-specific monitor of CNS white matter *in vivo*. Its decrease could represent either an irreversible process of axonal loss or a potentially reversible axonal metabolic dysfunction, while an eventual increase of NAA levels can be found in neuronal adaptation (Bjartmar *et al*., 2002).

When applied to the study of multiple sclerosis, a decrease in NAA—both for absolute values and normalized to creatine (Cr)—has been found not only in demyelinating lesions (Matthews *et al*., 1991; Arnold *et al*., 1992), but also in the normal-appearing white matter (NAWM) (De Stefano *et al*., 1998; Fu *et al*., 1998). A decrease in NAA in the NAWM has also been found in multiple sclerosis of the relapsing-remitting type (RRMS) at early stages of the disease and with minimal clinical disability (Chard *et al*., 2002; Casanova *et al*., 2003), or even in patients with a particularly low demyelinating lesion load and no significant disability (De Stefano *et al*., 2002). These metabolic changes suggest pathological processes that precede the MRI-defined lesion and, therefore, it seems valuable to study their relevance in relation to initial clinical aspects of the disease (Wolinsky and Narayanan, 2002). A correlation between functional disability, measured with the Expanded Disability Status Score (EDSS) (Kurtzke, 1983) and NAA levels has been reported for the more disabled RRMS patients (De Stefano *et al*., 1998), but the exact significance of the NAA decreases in patients without clinically significant disability remains unclear. Some authors have suggested that it could represent an index of diffuse axonal injury that would begin at disease onset, but remain clinically silent due to compensatory brain mechanisms (Reddy *et al*., 2000). Therefore, it is of relevance to explore the possible association between NAA decreases when present with other clinical domains relative to multiple sclerosis that are not measured by the standard scales which address functional disability.

It is widely accepted that multiple sclerosis has an impact on cognitive function, mainly for attention, memory and executive functions, although with considerable heterogeneity for each patient profile (Rao *et al*., 1991; Amato *et al*., 1995). Moreover, when restricting the study to the more benign form of the disease (RRMS), from 24% up to almost 50% of the patients suffer from some kind of attentional deficit (De Sonneville *et al*., 2002; DeSousa *et al*., 2002; Rovaris *et al*., 2002). Attention is a broad term used to designate the processes that mediate the appropriate allocation of physiological/cognitive resources to relevant stimuli, referring these stimuli to objects, locations or moments (Coull, 1998). Attentional processes are modulated by, and also modulate, the intrinsic arousal level—the term arousal referring to the physiological reactivity of the subject with regard to behavioural processes (Robbins and Everitt, 1995). The neural correlates of the attention-arousal network are thought to integrate two main cortical systems, i.e. the posterior parietal and the anterior frontal including the cingulate, with subcortical structures—the thalamus and the ascending reticular activating system (ARAS) of the brain (Posner and Petersen, 1990; Mesulam, 1999). The ARAS, located along the brainstem, consists essentially of a series of nuclei each containing neurochemical cell bodies whose axons innervate large areas of cortex. Recent animal (Aston-Jones *et al*., 1999) and human (Coull *et al*., 1999) studies have linked these pathways, especially the noradrenergic originated at the locus coeruleus, to the modulation of attention.

Considering that the attentional function seems to depend on a broad network whose origin would start at the ARAS, we hypothesized that a marker of brain injury—such as NAA decreases—by the fourth ventricle in the pons (corresponding approximately to the location of the locus coeruleus, Nestler *et al*., 1999) would correlate with worse attentional performance in RRMS patients. To investigate this, we chose the dichotic listening (DL) task (binaural competitive stimulation) with verbal material because it can be presented in a manner that focuses on linguistic lateralization (classical or 'non-forced') or include specific instructions to pay attention to a given ear, a condition that isolates the attentional process better (Hugdahl, 2000). In this latter condition, called 'forced attention', the subject must identify one verbal item while simultaneously inhibiting the competitive one delivered to the opposite ear, thus the type of attention embraced is basically selective or focused (Coull, 1998). For the present study, we designed a DL tape in which a tone cue presented before each dichotic trial indicated the item to be attended, so the subject had to shift his attention from trial to trial. This procedure—an exogenous cue as opposed to the endogenous cue where one verbal instruction is given for the rest of the test—is currently the best way to automatically capture the attention of the subject for the auditive domain (Mondor and Bryden, 1991). A homogeneous RRMS sample with mild physical disability at early stages of the disease performed significantly worse for this forced attention task when compared with a matched control group (Gadea *et al*., 2002).

Our main goal was to analyse the relation between NAA levels measured at the pons and the forced attention DL test

performance in an early RRMS sample with mild disability and no demyelinating lesions within the brainstem. We expected that the patients with the lowest NAA/Cr levels would exhibit the worst attentional performance. Moreover, studies applying functional neuroimaging have demonstrated higher right hemisphere metabolic activation when performing a variety of attentional tasks (for a review, see Coull, 1998); thus, we sought to explore whether this laterization mirrors the integrity of ARAS projections by measuring axonal damage in both hemipons.

Methods

Subjects

From a population of 40 RRMS patients included in an inter-hospital clinical trial, we selected a sample providing baseline evaluations meeting strict clinical, radiological and perceptual criteria of interest. Finally, 19 RRMS patients, nine males and ten females, were included. They met the criteria of Poser *et al*. (1983) for RRMS. Their disease evolution was <5 years and the maximum EDSS score was 3.5. Table 1 shows demographical and clinical characteristics for the sample. None of the patients had demyelinating lesions in the area of interest studied, although all of them had cerebral supratentorial lesions. ^{1}H-MRS was carried out at least 3 months after the last exacerbation. The cognitive measures were taken within a 15-day interval from the MR scanning. The patients were included if they were right-handed (Castresana *et al*., 1989) and met the audiometric criterion for inclusion (<10 dB ear difference at 500, 1000, 2000, 3000 and 6000 Hz). All patients were in clinical remission at the time of the study. Informed consent according to the Declaration of Helsinki was obtained from all patients. The IRB/IEC of La Fe University Hospital approved this study.

^{1}H-MRS

After imaging with turbo spin echo T2 and proton density weighted images [TR/TE (repetition time/echo time) 5154/13 and 115 ms], spectroscopy experiments were acquired with two dimensional turbo-spectroscopic imaging sequences (2DTSI) in a 1.5 T Philips Gyroscan Intera NT (Philips Medical Systems, The Netherlands) using a 90°–180°–180° pulse sequence. The anterior and posterior commissures (AC–PC) were used to locate the region of interest in a plane parallel to AC–PC line at pons, just at the fourth ventricle. Signals from surrounding cortical and bone areas were suppressed with 12 saturation slabs perpendicular to the acquisition plane (see Fig. 1). Shimming and tuning were achieved with automated procedures before acquisition. Water signal was suppressed with selective excitation. The multivoxel spectroscopic image matrix consisted of a grid of 24 × 24 elements (576 voxels) with a field of view of 230 × 230 mm and a slice thickness of 20 mm. Each volume unit dimension was 9.6 × 9.6 × 20 mm (1.8 ml). The experiments were acquired with long TE time (272 ms) and a TR of 2000 ms. A total of 256 points were collected with a spectral width of 1050 Hz through 128 averages. Data processing included Gaussian and exponential broadening for filtering, zero filling to 512 points and Fourier transformation.

A set of non-suppressed water spectra was also collected in the same sequence acquisition with a lower resolution (a matrix of 12 × 12 with voxels of 19.2 × 19.2 × 20 mm, 7.35 ml) as a reference in order to automatically correct the phase over the 2DTSI grid. The water resonance data in the reference set of spectra were used in the reconstruction for correction of chemical shift and amplitude differences due to magnetic field inhomogeneity. Spectra quality was controlled through the shape, the width at half height, and the intensity of the water resonances measured with jMRUI software (Naressi *et al*., 2001). These methodological control parameters remained constant for all ^{1}H-MRS studies, indicating a stable magnetic field homogeneity (Casanova *et al*., 2003).

From the whole matrix of the 2DTSI slice, two points were selected at the right and left hemipons sides by the theoretically expected locus coeruleus location (see Fig. 2). Both spectra were Fourier transformed and analysed using the jMRUI software. Absolute spectra were used in order to overcome first-order phase distortions associated with turbo spectroscopy imaging pulse sequence artefacts. Therefore, time domain fitting by jMRUI was not used to integrate the spectra. The height of the peaks was used instead of the areas as the magnetic field homogeneity, similar water line width and T2 values for any metabolite were constant (Danielsen and Ross, 1999). Cr concentration was assumed to remain unchanged (Arnold *et al*., 1998); the ratio of NAA/Cr, considering Cr as an internal reference, was used for calculations.

Table 1 *Demographical, clinical, cognitive and biochemical characterization of RRMS patients*

Variables	Whole sample	Impaired attention	Preserved attention
Demographical			
Sex (M/F)	9/10	3/6	6/4
Education (years)	12.6 ± 3.4 (8–17)	11.4 ± 3.6 (8–17)	13.8 ± 3.1 (8–17)
Age	24.5 ± 4.1 (19–32)	25.5 ± 3.9 (19–31)	23.7 ± 4.4 (19–32)
Clinical			
EDSS [(range), median]	(0–3.5), 1	(1–3.5), 2	(0–2), 1
Evolution (months)	24 ± 14 (10–60)	29.6 ± 16.8 (12–60)	18.9 ± 8.9 (10–36)
Cognitive			
LI	35.2 ± 20.4 (0–75.9)	40.6 ± 20.6 (8–65.4)	30.9 ± 20.1 (0–75.9)
ASI	1.5 ± 1.2 (–0.09 to 3.5)	0.5 ± 0.4 (–0.09–1)	2.6 ± 0.7 (1.66–3.5)
Biochemical			
Global NAA/Cr	4.7 ± 1 (2.7–6.6)	4 ± 0.8 (2.7–5.4)	5.3 ± 0.8 (3.8–6.6)
Right NAA/Cr	4.6 ± 1.3 (2.1–7.6)	3.8 ± 0.8 (2.1–4.7)	5.3 ± 1.3 (3.5–7.6)
Left NAA/Cr	4.8 ± 1 (3–6.4)	4.2 ± 1 (3–6)	5.2 ± 0.9 (3.3–6.4)

All variables except for EDSS expressed as mean ± SD (range).

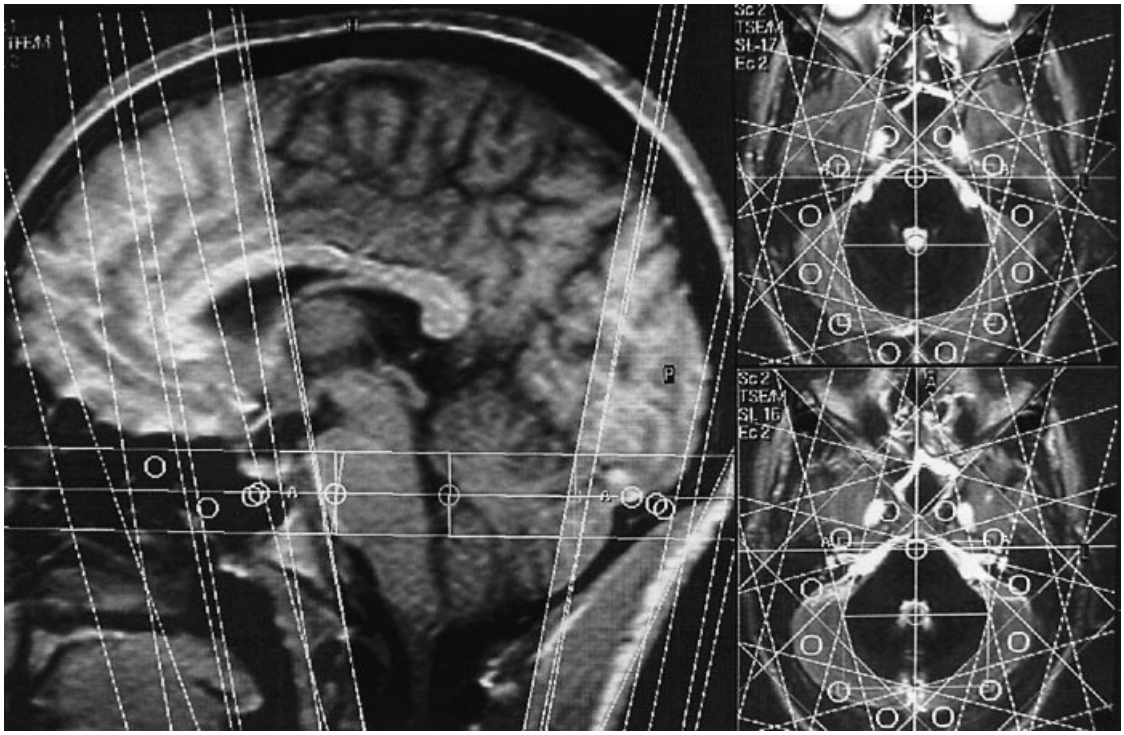

Fig. 1 Slice locations for ^{1}H-MRS of the cerebellum and brainstem, overlaid on midsagittal (left) and transversal (right) T1 and T2-weighted localizer images. The 12 saturation slabs perpendicular to the acquisition plane are also shown.

DL tests

Tape 1: DL without attentional instructions (classical or non-forced)

The dichotic stimuli consisted of the six stop consonants paired with the vowel 'a' to form six consonant vowel syllables (ba, da, ga, ka, pa, ta). Thirty dichotic syllables were generated and then duplicated and recorded randomly, giving 60 test trials with a maximum correct score of 60 (for details, see Gadea *et al*., 2000). The test was scored applying the single correct method (asking the subject to report only one syllable for each trial; Bryden *et al*., 1983).

Tape 2: DL with forced attention

The dichotic tape as described above was digitally manipulated to add a tone to one ear or the other prior to each trial. This tone was designed to cue subjects to focus attention on the ear in which it sounded. The interval between the onset of the tone and the onset of the stimuli was 450 ms. This interval was chosen because it magnifies the attentional effect (Mondor and Bryden, 1991). The tone was presented 30 times cueing the right ear and 30 times cueing the left ear (all possible combinations for each ear). The presentation order was random, but with the constraint that one ear was cued no more than four successive times. This allowed control over the vagaries of complete randomization without compromising essential unpredictability. Subjects were asked to report only the consonant–vowel presented to the ear in which the cue had sounded. The maximum correct score was 30 for each ear. The hits (cued syllable correctly reported) and the intrusions (non-cued syllable reported) for each ear were computed.

The presentation order for the tapes was fixed: tape 1 (classical or non-forced DL) was always given before tape 2 (forced attention). Both tapes were replayed to the patients from a Sony Walkman WM-EX1HG mini cassette player with plug-in type Sennheiser HD545 headphones. The output from the cassette player was calibrated at a level of 75 dB.

Data management

To analyse the biochemical variables, two different sets of data were taken into account: (i) the biochemical ratios obtained from the two selected regions of the pons, Right NAA/Cr and Left NAA/Cr, as contributions from each locus coeruleus; and (ii) the average of these two points as a Global NAA/Cr evaluation of that structure.

To analyse the DL performance, the raw scores obtained for each of the two DL tapes were transformed into the percentages of correct syllables according to their maximum score [note that tape 1 (classical) had a maximum score of 60, while tape 2 (attentional) had a maximum score of 30, due to cueing half of the dichotic items to each ear]. This allowed the comparison between the DL test without attention and the one with forced attention instructions (Gadea *et al*., 2002).

Additionally, a composite index was obtained for each tape, as follows:

For the classical DL test (tape 1), a laterality index (LI) score was calculated according to the formula:

$$LI = [(RE - LE)/(RE + LE)] \times 100$$

where RE (right ear) and LE (left ear) are the number of correct syllables from the right and left ear, respectively.

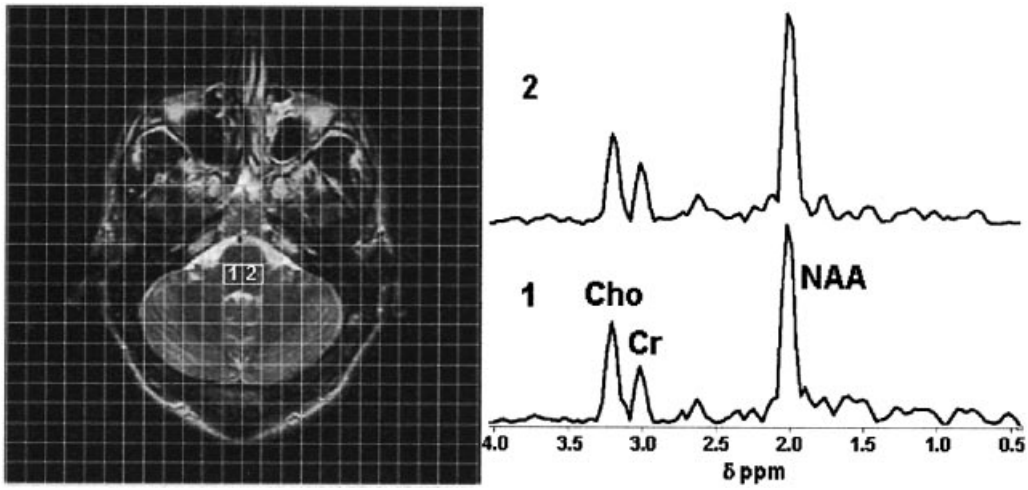

Fig. 2 Spatial location, visualization and analysis of the ^{1}H-MRS imaging of the right (1) and left (2) regions of the pons. High field homogeneity allows complete resolution of NAA and Cr resonances, with a minimal lipid contamination in both regions through efficient use of the saturation slabs. Cho = choline.

For the forced attention DL test (tape 2), an attentional shift index (ASI) was calculated according to the formula:

$$ASI = \ln [(REFR \times LEFL)/(LEFR \times REFL)]/ \sqrt{[(1/REFR) + (1/LEFL) + (1/LEFR) + (1/REFL)]}$$

where REFR and LEFL are the number of hits from right and left ear respectively, whereas REFL and LEFR are the number of intrusions from right and left ear, respectively. The ASI is based on a log transformation of the odds ratio of hits to intrusions. Due to the log-odds transformation, the index is not sensitive to the numbers of trials or to the number of responses given. Moreover, since an individual error term can be calculated, the magnitude of the attentional shift for each subject can be estimated. Values >1.645 indicate a significant attentional shift, while an inability to shift attention would be indicated by smaller values of ASI (for a detailed mathematical explanation of the ASI, see Asbjornsen and Bryden, 1998).

Statistical analyses

Both the biochemical and cognitive variables were normally distributed (Kolmogorov-Smirnov, $P > 0.05$). Regarding biochemical variables, a Student's *t*-test for dependent samples was applied to explore differences between right and left NAA/Cr hemipons levels. Differences in the DL percentages were explored by applying an ANOVA (analysis of variance) according to design 2 ('ear': RE versus LE) × 3 ['condition': non-forced (NF) versus forced right (FR) versus forced left (FL)]. The sphericity assumption for the three-level factor and its interactions was tested and confirmed by the Mauchly test ($P > 0.05$). *Post hoc* comparisons were performed by applying a Student's *t*-test for dependent samples. When testing associations between all the measured variables (clinical, cognitive, biochemical), Spearman correlations were obtained first. Then a multivariate stepwise linear regression model was applied to highly significant and normally distributed biochemical and cognitive variables. Finally, in order to explore differences between subgroups of patients based on the ASI cut-point, the Mann–Whitney *U* test with asymptotic significance (2-tailed corrected for ties) was performed for clinical and demographical variables (EDSS, months of evolution, years of education, age) while, for biochemical measures, a univariate ANOVA with variance homogeneity for subgroups confirmed by the Levene test was employed.

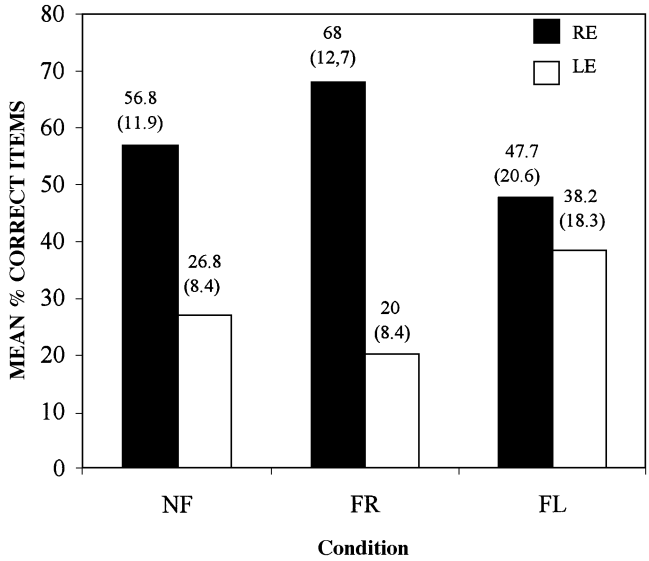

Fig. 3 Mean percentage of correct DL scores for both ears and conditions with SDs. All the comparisons showed significant differences except for RE to LE when performing the FL condition (see text).

Results

NAA/Cr levels

Table 1 shows the range, means and SD for the biochemical variables Left NAA/Cr, Right NAA/Cr and Global NAA/Cr for the whole sample of patients. The Student's *t*-test for

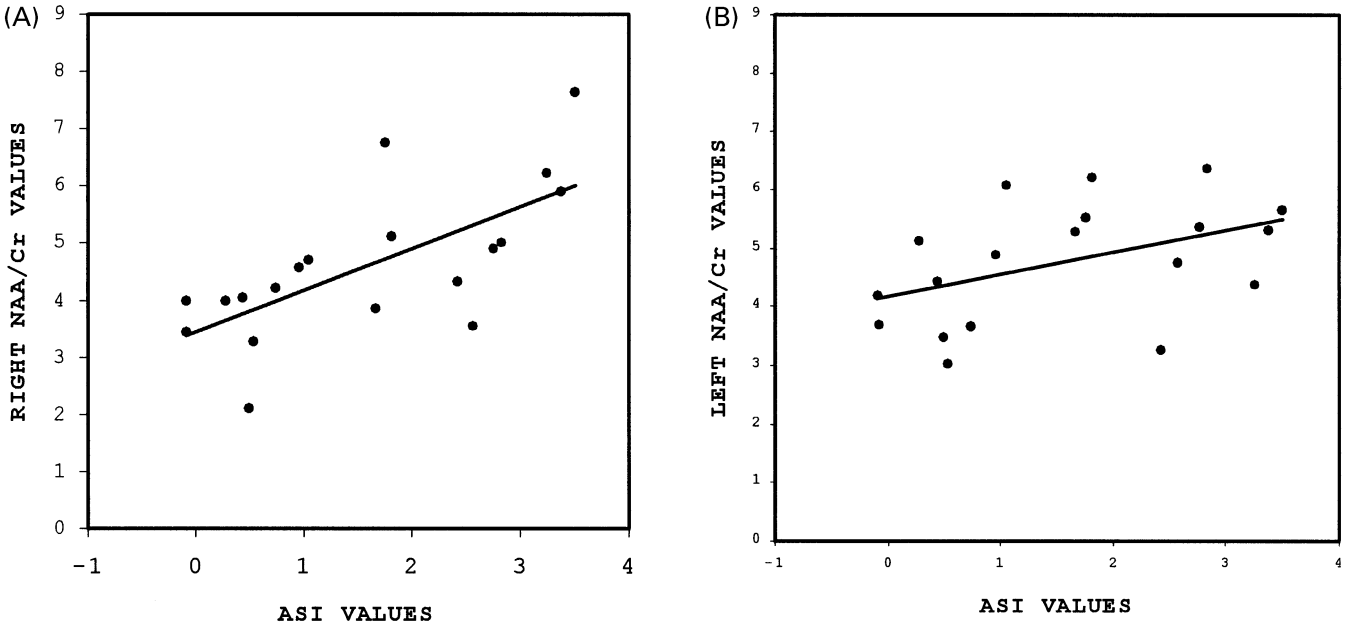

Fig. 4 (**A**) Scattergram for the linear regression between NAA/Cr levels measured at the right hemipons and the ASI values obtained from the RRMS patients (corrected $r^2 = 0.43$; $\beta = 0.68$; $P < 0.001$). (**B**) Scattergram for the linear regression between NAA/Cr levels measured at the left hemipons and the ASI values obtained from the RRMS patients. Note that the regression did not reach significance (corrected $r^2 = 0.15$; $\beta = 0.44$; $P < 0.06$).

dependent samples showed no differences [$t(18) = -0.62$, not significant] between Left NAA/Cr and Right NAA/Cr levels. Note that Table 1 also includes the biochemical variables split by the ASI cut-point. The Right NAA/Cr levels showed a non-significant lower mean than the Left NAA/Cr levels [$t(8) = -1.94$, $P = 0.08$] only for the group of attentional-impaired patients. There were no significant correlations between biochemical (Global NAA/Cr, Right NAA/Cr, Left NAA/Cr) and clinical (EDSS, months of evolution) measures.

DL performance

ANOVA

Fig. 3 shows DL performance for both ears in each of the three conditions tested (NF, FR and FL). The main effect of 'ear' was significant [$F(1,18) = 33.75$, $P < 0.0001$], indicating a greater overall RE mean. There were no differences in the global scores between conditions since the main effect of 'condition' did not reach significance [$F(1,18) = 2.21$, non significant). These effects were detailed by the significant interaction 'ear × condition' [$F(1,18) = 18.41$, $P < 0.0001$]. *Post hoc* comparisons showed that all the means were significantly different among them (t obtained from 4.59 to 10.26), except for the comparison between RE and LE scores when performing the FL condition [$t(gl18) = 1.09$, not significant]. Thus, the patients obtained a right ear advantage when performing the NF and FR conditions, but showed no significant differences between ears under the FL condition (see Fig. 3).

Analysis of the composite indexes LI and ASI

When exploring the LI obtained from the classical DL test, it was seen that none of the patients showed a left ear advantage (LI scores between 0 and 75.86). The correlation between LI and ASI scores was not significant. The patients as a group showed a mean below the cut-point of ASI scores (1.59, SD 1.21). In exploring the data for each patient, nine (47.3%) showed an ASI value below the cut-point, indicating an inability to shift attention. There were no significant correlations between cognitive (LI, ASI) and clinical measures (EDSS, months of evolution). Table 1 shows the range, means and SDs for these cognitive variables. These are shown for the whole sample and also divided into two groups based on the ASI cut-point (impaired attention versus preserved).

Correlations: global NAA/Cr levels and DL performance

Classical DL test

Spearman correlations showed no significant association between RE or LE or the composite LI scores with Global NAA/Cr levels ($\rho = 0.15$ or less, not significant).

Forced attention DL test

Spearman correlations for hits of each ear and Global NAA/Cr levels were positive and significant for the LE ($\rho = 0.44$, $P < 0.05$; ρ RE = 0.14, n.s.). Intrusions yielded

non significant negative correlations (LE ρ = –0.17; RE ρ = –0.28). The correlation for the composite ASI scores was significant, positive and larger (ρ = 0.66, $P < 0.002$), so a linear regression was applied with Global NAA/Cr on the dependent ASI scores. The amount of the ASI variability explained significantly (corrected r^2) by Global NAA/Cr was 39% [$F(1,18) = 12.9$, $P < 0.002$, β = 0.65].

Correlations: right versus left NAA/Cr levels and ASI scores

Classical DL test

Spearman correlations again showed no significant association with RE or LE or LI scores with any of the two hemipons NAA/Cr levels measured (ρ = 0.17, not significant).

Forced attention DL test

Correlations for hits of each ear and Right NAA/Cr levels were positive and significant for LE (ρ = 0.45, $P < 0.04$; RE ρ = 0.15, not significant). Intrusions and Right NAA/Cr levels were negative, although they did not reach significance (LE ρ = –0.25, RE ρ = –0.38). Exploring hits and Left NAA/Cr levels also yielded positive correlations without significance (LE ρ = 0.36, RE ρ = 0.12); for intrusions, the correlations with Left NAA/Cr were negative and non-significant (LE ρ = –0.04, RE ρ = –0.19). Thus, the analysis showed positive correlations for hits and NAA/Cr, and negative correlations for intrusions and NAA/Cr. The highest correlations were seen with Right NAA/Cr levels and LE hits (0.45) and RE intrusions (–0.38), i.e. with the performance under the FL condition—although significance was reached for LE hits only.

As in the case of the global evaluation of the pons, the situation changed when considering the composite ASI score. The correlation of ASI with Right NAA/Cr was highly significant (ρ = 0.72, $P < 0.0001$) and with Left NAA/Cr it was also significant although lower (ρ = 0.50, $P < 0.02$).

Finally, a multiple stepwise linear regression model was designed with Right NAA/Cr and Left NAA/Cr as the independent variables on the dependent ASI scores. Only Right NAA/Cr was accepted by the model (with probability to enter $F = 0.050$ and to remove $F = 0.100$). This variable significantly [$F(1,18) = 15.07$, $P < 0.001$, β = 0.68] accounted for 43% (corrected r^2) of the ASI variability (see Fig. 4A). The scattergram in Fig. 4B can also be consulted for the relationship between Left NAA/Cr levels and ASI scores. Note that this is presented mainly for illustrative purposes since Left NAA/Cr levels were not accepted by the designed stepwise model and, in fact when considering this relationship in a separate regression, it did not reach significance [$F(1,18) = 4.26$, $P = 0.06$, β = 0.44 for 0.15 corrected r^2).

Dichotomic analyses based on impaired attention

As the patients subdivided naturally into two groups based on performance for the attentional task, we attempted to identify differences between them. Table 1 shows that there were no differences with regard to demographical variables (sex, years of education or age). With respect to clinical variables, the impaired patients showed a higher mean for months of disease evolution and EDSS scores, but this only approached significance for EDSS scores ($Z = –1.70$, $P = 0.08$). Regarding biochemical variables, the difference between groups was highly significant for Global NAA/Cr levels [$F(1,18) = 9.86$, $P < 0.006$; confidence intervals for ASI impaired versus non-impaired 3.43/4.66 and 4.63/5.90, respectively], as well as for Right NAA/Cr levels [$F(1,18) = 9.18$, $P < 0.008$; confidence intervals 3.20/4.42 and 4.40/6.26, respectively]. For Left NAA/Cr levels, the difference was also significant but with lower statistical power [$F(1,18) = 4.64$, $P < 0.04$; confidence intervals 3.55/5.02 and 4.56/5.86, respectively].

Discussion

This study has many singularities and some implications should be pointed out. First, we looked specifically for NAA/Cr levels in the NAWM of early RRMS patients with low disability and no lesions at the brainstem. De Stefano *et al*. (2002) showed that cerebral NAWM NAA/Cr is diffusely decreased in multiple sclerosis patients with early disease, low demyelinating lesion load and no significant disability. In our series, NAA/Cr levels did not relate to disability (EDSS) or to the disease duration expressed in months. On the other hand, the possibility of NAA/Cr decrease being related to other pathological features of the disease, like cognitive loss, has not been fully explored in the literature. To our knowledge, there is only one study that explicitly looked for relationships between NAA levels and cognitive function in multiple sclerosis (Pan *et al.*). These authors found a correlation between memory (Selective Reminding Test) and executive (Tower of Hanoi Test) functions, with NAA levels at the periventricular white matter where demyelinating lesions are usually located—thus making it unclear why NAA decreases in this particular region relate to those tests (or functions) and not to others. In this study, we assumed a functional–anatomical oriented guide with the aim of correlating cognitive biological features that theoretically should be so, and trying to connect the axonal damage as the most basic structure relevant to attention, the ARAS (Posner and Petersen, 1990; Mesulam, 1999), with actual performance in an attentional task.

The DL test was selected to check for attentional deficits in RRMS patients in our series. The Paced Auditory Serial Addition Test (PASAT) is a well-known measure of information speed processing and was introduced in the clinical practice as a part of the Functional Composite Scale (Cutter *et al*., 1999); however, it is not related to NAA levels (Chard

et al., 2002). Moreover, the PASAT, or at least its standard scoring, has been recently criticised by Fisk and Archibald (2001). On the other hand, the DL paradigm with attentional instructions has been applied to the study of various pathologies such as schizophrenia, depression, dementia and learning difficulties (Claus and Mohr, 1996; Obrzut *et al*., 1997; Loberg *et al*., 1999; Hugdahl *et al*., 2003). In multiple sclerosis evaluation, the DL test was introduced basically in its classical form (thus, without attentional instructions or non-forced) to test for hemispheric disconnection due to corpus callosum loss (Rao *et al*., 1989; Pelletier *et al*., 2001) and has only occasionally been applied to test for attentional deficits as well (Reinvang *et al*., 1994; Gadea *et al*., 2002). In this study, the analysis of the performance for the three conditions (NF, FR and FL) demonstrated that the patients as a group showed the ability to modify the scores from an attention-free situation (NF) to one with forced attention, but this was not sufficient to reach a left ear advantage when given the FL instructions. This pattern is similar to that observed in schizophrenic patients (Hugdahl *et al*., 2003). It has been suggested that this indicates a deficit in attentional–executive functions, since a general finding in groups of healthy individuals is that the right ear advantage usually obtained for the NF condition is increased when attention is shifted to the RE stimulus, and switched to a left ear advantage when attention is shifted to the LE stimulus (for an overview, see Hugdahl, 1995).

Moreover, in this study, the raw scores obtained for both forced attention conditions (hits and intrusions from each ear) were integrated in an ASI score. The ASI score offers the advantage of a cut-point, important when DL is employed in clinical screening for attentional deficits and in clinical studies on populations where attentional problems are expected (Asbjornsen and Bryden, 1998). In our sample, almost half of the patients showed attentional dysfunction. When attempting to differentiate the clinical status of the patients based on the ASI cut-point, we found a tendency for the attentional-impaired patients to be more disabled as measured with the EDSS. It has been suggested that cognitive decline is, in part, independent of functional disability (Kujala *et al*., 1997). It should be noted that, in an earlier report, there was a significant difference between multiple sclerosis patients and controls regarding ASI scores, although 20% of the control group also showed a mean under the cut-point (Gadea *et al*., 2002). Asbjornsen and Bryden (1998) found all their sample of dyslexic children attentionally impaired on the basis of the ASI cut-point, but 50% of normal control children were also impaired. Other researchers have reported about 20% of healthy adults who had to be excluded from the DL task for the same reason, mainly due to difficulties when performing the FL condition; they have argued that the difficulty of the forced attention DL task could explain such a peculiarity (Mondor and Bryden, 1991). We suggest that the DL paradigm may be tapping the highest human attentional capacities or resources, which are not equal in all subjects from a healthy population. This feature is reinforced by the finding of ageing increasing the possibility for a worse DL attentional test performance (Beaton *et al*., 2000). Nevertheless, it is repeatedly observed that, in RRMS, schizophrenia or severe dyslexia, there is a significantly higher proportion of individuals attentionally impaired in this task compared with controls. We would like to point out that some unknown disease mechanisms are affecting those higher attentional capacities.

The main finding of this study was that early RRMS patients with greater selective attention deficits showed a lower level of NAA/Cr in the NAWM located at the right pontine ARAS, closely involving the locus coeruleus or its projections. The exact significance of NAA decrease in RRMS is the focus of current research. In a recent report (Filippi *et al*., 2003), the concentration of whole-brain NAA was quantified in a group of RRMS patients at the earliest clinical stage of the disease and was found to be significantly lower in them compared with the controls. The authors concluded that widespread axonal pathology, being independent of MRI-visible inflammation and too extensive to be completely reversible, argues against the hypothesis that axonal pathology of multiple sclerosis is the final result of repeated inflammatory events, and in favour of early neuroprotective intervention. From the present data and because RRMS patients with greater attentional disturbances exhibited the lowest NAA/Cr levels (both from a continuous point of view and from a dichotomic one based on the ASI cut-point), it is concluded that NAA provides a specific measure of axonal damage relevant for the cognitive status of early stage RRMS patients.

A noticeable finding was that pontine NAA/Cr levels did not correlate with the LI, which has implications for laterality research in general and for studies on dichotic listening. Hugdahl (1995) proposed a model of DL performance in which a stimulus-driven or bottom-up processing would be measured through the classical version of the DL test, being more related to interhemispheric connectivity and linguistic lateralization. This would be modulated by an instruction-driven or top-down processing measured through the forced attentional shift version of the DL test, which would represent the dynamic modulation of attention to either hemispace. Following this suggestion, the classical, but not the forced attention DL test, must be correlated with corpus callosum atrophy measures, as was demonstrated in an earlier report (Gadea *et al*., 2002). Likewise, the forced attention DL test, but not the classical DL test, must be linked to deficits in attention-related structures, as in the present study regarding the ARAS. The additional observation of no significant correlation between the LI and the ASI could be because the two measures are partially independent. Moreover, we explored the correlations of NAA/Cr with each of the five variables obtained from the forced attention DL test (four raw scores representing hits and intrusions from each ear, and one composite ASI obtained from them). The composite ASI was the variable more closely related to NAA/Cr levels when measured near the pontine locus coeruleus nuclei of the

ARAS. As the ASI reflects a global aspect of selective attention, related to both attending to the right and left hemispace, we point out the possibility that the pontine ARAS system, being the most basic structure of the attentional brain network, could be more closely linked to the general selective attention resources. Functional MRI has demonstrated that the forced DL test increased activation in higher cortical structures related to attention (right posterior and inferior superior parietal lobe) compared with the classical DL test (Hugdahl *et al*., 2000). It would be of interest to test a replication of this study with NAA levels in comparable regions.

Moreover, the present study also has implications for research on attention neurobiology and its lateralization. From both a continuous and a dichotomic point of view, the analyses of our data mainly implicated the right hemipons in selective attention. Functional imaging studies (PET) in humans performing a visual reaction time task without warning stimulus have shown a thalamic-brainstem activation (Kinomura *et al*., 1996) preferentially for the right side (Sturm *et al*., 1999), thus connecting these areas with intrinsic alertness (the most basic aspect of attention). A rapid visual information processing task combined with noradrenergic pharmacological treatment has also shown increased connectivity from right locus coeruleus to parietal cortex in another PET study (Coull *et al*., 1999). Electrophysiological studies recording the firing of locus coeruleus cells in monkeys performing visual discrimination tasks have concluded that the phasic mode of locus coeruleus activity may promote focused or selective attention (for a review of these studies, see Aston-Jones *et al*., 1999). Although it used a different methodology, this study also relates the right ARAS (possibly involving the locus coeruleus) to auditive selective attention.

Finally, several recent reports have shown that, apart from the macroscopic lesions as the obvious pathological multiple sclerosis feature, significant axonal damage might occur within the more extensive NAWM ('the axonal hypothesis'). We would like to subscribe to the argument that a more demanding test is the demonstration of a graded relationship between specific markers of axonal loss and impairment within a single functional system (Lee *et al*., 2000). These authors found lower NAA levels in the NAWM of the internal capsule related to upper-limb motor impairment in multiple sclerosis, and suggested that lesions anywhere within a pathway may act on spatially remote levels of NAA via degenerative processes (e.g. Wallerian degeneration). Given that the ARAS system projects diffusely to virtually all the cerebral cortex and that in our sample all patients had cerebral supratentorial lesions, we should consider the possibility of a subjacent remote degenerative process affecting the pontine ARAS system.

In conclusion, by selecting a group of early RRMS patients and by assessing NAA/Cr in the pontine NAWM and the performance in a complex attentional task, it was demonstrated that the impairment of selective attention increases with the degree of axonal damage at the pons (mainly on the right side), possibly due to neurodegeneration of locus coeruleus cells or its projections.

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