Published in final edited form as:

Eur Radiol. 2018 March ; 28(3): 1140–1148. doi:10.1007/s00330-017-5064-9.

Reduced GABA levels correlate with cognitive impairment in patients with relapsing-remitting multiple sclerosis

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Abstract

Objectives—To investigate if brain gamma-aminobutyric acid (GABA) levels in patients with relapsing-remitting multiple sclerosis (RRMS) are abnormal compared with healthy controls, and their relationship to cognitive function in RRMS.

Methods—28 RRMS patients and 26 healthy controls underwent magnetic resonance spectroscopy (MRS) at 3 T to detect GABA signals from posterior cingulate cortex (PCC), medial

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Guarantor:

The scientific guarantor of this publication is Guangbin Wang.

Conflict of interest:

The authors of this manuscript declare no relationships with any companies, whose products or services may be related to the subject matter of the article.

Statistics and biometry:

No complex statistical methods were necessary for this paper.

Compliance with ethical standards:

Informed consent:

Written informed consent was obtained from all subjects (patients) in this study.

Ethical approval:

Institutional Review Board approval was obtained.

Methodology:

- prospective
- case-control study
- performed at one institution

prefrontal cortex (mPFC) and left hippocampus using the “MEGAPoint Resolved Spectroscopy Sequence” (MEGA-PRESS) technique. All subjects also underwent a cognitive assessment.

Results—In RRMS patients, GABA+ were lower in the PCC ($p = 0.036$) and left hippocampus ($p = 0.039$) compared with controls, decreased GABA+ in the PCC and left hippocampus were associated with specific cognitive functions ($r = -0.452$, $p = 0.016$ and $r = 0.451$, $p = 0.016$ respectively); GABA+ in the mPFC were not significantly decreased or related to any cognitive scores ($p > 0.05$).

Conclusions—This study demonstrates that abnormalities of the GABAergic system may be present in the pathogenesis of RRMS and suggests a potential link between regional GABA levels and cognitive impairment in patients with RRMS.

Keywords

Multiple sclerosis; GABA; ^1H -MRS; MEGA-PRESS; Cognitive impairment

Introduction

Cognitive impairment is a major disabling feature of multiple sclerosis (MS), affecting 40% to 70% of patients [1], with information processing speed, immediate and delayed recall or memory, executive function, attention, and visuospatial abilities the most commonly affected functions [2]. The pathological processes underlying these cognitive impairment are complex, involving molecular-level changes including abnormal neurometabolite levels [3], disruption of the integrity of brain microstructures [4, 5], and macroscopic abnormalities to structural pathways and functional cognitive networks.

Amongst the neurometabolites of interest, gamma-aminobutyric acid (GABA), the principal inhibitory neurotransmitter in the brain, involved in neural plasticity and the generation of synchronised network oscillations, is crucial for effective information processing in the brain [6] and normal cognitive function [7, 8]. Levels of GABA *in vivo* can be measured non-invasively using magnetic resonance spectroscopy (MRS). However, measurements of GABA using conventional ^1H MRS *in vivo* have limited accuracy due to GABA's relatively low concentration and spectral overlap with stronger signals of other major metabolites. Edited MRS, using e.g. Mescher-Garwood point resolved spectroscopy (MEGA-PRESS) [9], allows selective detection of GABA [10] and has been successfully applied in a number of neurologic and psychiatric disorders [11–13], as well as in healthy control subjects [14–16]. [17][18] Recent MRS studies have detected GABA levels in patients with progressive multiple sclerosis [17] and relapsing-remitting multiple sclerosis (RRMS) [18], and they have focused on the role of GABA in physical disability in this disease. However, to date there have been few studies exploring the relationship between GABA levels and cognitive function in patients with MS.

Therefore, in this study, the MEGA-editing technique was used to measure the levels of GABA in the posterior cingulate cortex (PCC), medial pre-frontal cortex (mPFC) and left hippocampus, which have been identified as key areas in the default mode network in normal cognition [19, 20] and in cognitive processing of MS [21–23]. [21, 22][23] Further,

we will investigate whether the GABA levels are correlated with cognitive function in patients with RRMS.

Materials and methods

Subjects

According to the Declaration of Helsinki, all participants provided their written informed consent before entering the study, which was approved by the institutional review board of Shandong Medical Imaging Research Institute, Shandong University (2015-001).

Twenty-eight patients with RRMS (median Expanded Disability Status Scale [EDSS] [24], 2 [range, 0 – 4]) were recruited for this study. All the patients met the criteria for diagnosis of RRMS [25] and had no history of relapse and steroids treatment within the preceding 3 months. The exclusion criteria for patients were the following: 1) a history of head trauma, neurologic, or psychiatric disorder; 2) previous or current substance abuse; 3) intake of GABAergic agents (e.g. baclofen) before enrolment.

Twenty-six age- and gender-matched healthy controls (HCs) were enrolled. The exclusion criteria were as follows: 1) a history of head trauma, neurologic, or psychiatric disorder; 2) previous or current substance abuse.

All subjects were right-handed. Smoking, drinking alcohol and taking caffeine were prohibited for 12 hours prior to MR measurement.

Cognitive tests

All participants were asked to complete a set of standardised comprehensive cognitive tests. Information processing speed was assessed using the Symbol-Digit Modalities Test (SDMT) [26]. Executive function was measured using the Trail-making test (TMT) [26]. Verbal memory was assessed using the Rey Auditory Verbal Learning Test (RAVLT) for immediate and delayed recall [26]. Visuospatial function was assessed using the Rey-Osterich complex figure test [27]. Attention was assessed using the Stroop colour word interference test [28]. Raw scores were standardised (z-transformed) based on the mean and standard deviation (SD) of the HCs. Neuropsychological testing was overseen by a board-certified clinical neuropsychologist.

Imaging acquisition

All MRI and MRS scans were performed using 3 T Achieva system (Philips Medical Systems) with an 8-channel phased-array head coil. A T1-weighted three-dimensional turbo field echo (TFE) scan was acquired for MRS voxel localisation and subsequent tissue segmentation. The scanning parameters were as follows: repetition time (TR) = 8.1 ms; echo time (TE) = 3.7 ms; slice thickness = 1 mm; field of view = $24 \times 24 \text{ cm}^2$ flip angle = 8° ; voxel size = $1 \times 1 \times 1 \text{ mm}^3$. T2-FLAIR images (TR = 11000 ms; TE = 125 ms; slice thickness = 3 mm; field of view = $24 \times 20 \text{ cm}^2$; voxel size = $1 \times 1 \times 3 \text{ mm}^3$) were acquired in order to count number of brain lesions.

The dimensions for the MRS volumes of interest (VOI) were as follows: (1) posterior cingulate cortex (PCC): size $3 \times 3 \times 2 \text{ cm}^3$; (2) medial pre-frontal cortex (mPFC): size $2 \times 3 \times 3 \text{ cm}^3$; and (3) left hippocampus: size $4 \times 2 \times 2 \text{ cm}^3$, as shown in Figure 1. The median sagittal plane was selected as a reference slice for the voxel in PCC and mPFC: the PCC voxel was positioned posterior and superior to the splenium of the corpus callosum, aligned with the shape of the corpus callosum and repositioned on the medial aspect of the axial plane; the mPFC voxel was positioned with the posterior edge of the voxel parallel to the genu of the corpus callosum and repositioned on the medial aspect of the axial plane. The left hippocampus voxel was positioned parallel to the body of the hippocampus in a parasagittal section and mainly including parahippocampal. All of the VOIs were positioned in such a manner as to avoid the lateral ventricles and skull.

The MEGA-PRESS editing sequence was used for GABA detection, with parameters $TR = 2000 \text{ ms}$; $TE = 68 \text{ ms}$; 256 signal averages; acquisition bandwidth = 2000 Hz. During odd-numbered acquisitions, a frequency-selective, Gaussian inversion pulse was applied to GABA- $^3\text{CH}_2$ resonance at 1.9 ppm, affecting the weakly J-coupled triplet peak of GABA- $^4\text{CH}_2$ at 3.01 ppm (EDIT-ON). During even-numbered acquisitions, the same pulse was applied symmetrically to the other side of the water peak, at 7.5 ppm (EDIT-OFF). The GABA-edited spectrum was obtained from the difference between the EDIT-ON and EDIT-OFF spectra. Chemical Shift Selective Suppression (CHESS) was used for water suppression. FASTMAP shimming of the VOI was performed automatically before each acquisition. For quantification, a shorter measurement (4 averages) of the unsuppressed water signal from the same volume was obtained.

^1H -MRS post-processing

As the signal detected at 3.01 ppm is known to contain contributions from both macromolecules (MM) and homocarnosine [29], the detected signal is referred to as GABA+ rather than GABA. The GABA+ data were processed using the Gannet 2.0 toolkit in Matlab 2012b (Mathworks) [30]. Gannet uses a single-Gaussian model to fit the edited GABA+ peak, and a Gaussian-Lorentzian model for water signal. 3 Hz exponential line broadening was applied. GABA+ levels [GABA+] was evaluated relative to water amplitude in institutional units (IU). Gannet also provides normalised residual fitting errors of GABA+, which is used to assess spectral quality. Only spectra that had fitting errors of GABA+ below 15% were included in the statistical analysis.

3D T1-weighted brain images were segmented to calculate the fractional content of grey matter (GM), white matter (WM) and cerebrospinal fluid (CSF) within each spectroscopic voxel using an automatic brain segmentation program, FAST (FMRIB's automated segmentation tool) in the FSL package (Oxford University, Oxford, UK) [31]. VOIs were co-registered to the anatomical images using the "Re-creation of VOI" Matlab tool [32]. The concentration of GABA in CSF was assumed to be negligible.

Statistical analysis

Statistical analyses were carried out using the statistical package SPSS 17.0. All the results are quoted as mean $\pm$ SD values. The differences of GABA+ levels between patients and

controls were tested using an analysis of covariance (ANCOVA) with age as a covariate. For neuropsychological comparisons, a general linear model was used to adjust for age, gender and education (in years). Correlations between GABA+ levels and neuropsychological scores in RRMS were tested using the Pearson correlation coefficients (r). Partial correlation coefficients were also calculated, adjusting for age. The threshold for significance was $p < 0.05$.

Results

Demographic characteristics of study subjects

Demographic and clinical data are presented in Table 1. No differences in age, gender distribution and educational level were found between the groups. Compared with controls, patients had significantly worse verbal memory ($p = 0.002$), with worse immediate ($p = 0.006$) and delayed recall ($p = 0.001$). In addition, patients showed significantly slower processing speed on the SDMT ($p = 0.006$) and had worse executive function on TMT ($p = 0.002$) compared to the control group. By contrast, no difference between patients and controls was found in their visuospatial function ($p = 0.155$) or attention ($p = 0.585$).

Tissue segmentation and data quality

The mean GM tissue fraction GM/(GM + WM) for the RRMS group was 60.53%, 64.48% and 53.78% in the PCC, mPFC and left hippocampus, respectively, while it was 60.49%, 64.78% and 52.87% for the control group (Table 2). No significant difference was found between voxel GM tissue fractions (PCC, $p = 0.973$; mPFC, $p = 0.742$; left hippocampus, $p = 0.351$). The mean volume of GM for the RRMS group was 10.13 cm³, 10.35 cm³ and 8.25 cm³ in the PCC, mPFC and left hippocampus, respectively, while it was 10.13 cm³, 10.36 cm³ and 8.14 cm³ for the control group (Table 2). These were not significantly different (PCC, $p = 0.961$; mPFC, $p = 0.916$; left hippocampus, $p = 0.453$).

The fitting errors of GABA+ did not differ between RRMS patients and controls in the PCC ($p = 0.16$), mPFC ($p = 0.53$) and left hippocampus ($p = 0.365$) (Table 2).

The full-width at half-maximum (FWHM) of all VOIs were below 15 Hz in RRMS and control groups. The mean FWHM for the RRMS group was 7 Hz, 10.04 Hz, 12.07 Hz in the PCC, mPFC and left hippocampus, respectively, while it was 7.23 Hz, 10.38 Hz, 12.46 Hz for the control group (Table 2).

Comparisons of GABA+ levels between groups

One patient dataset was excluded from the mPFC due to the fitting error $> 15\%$. Representative GABA-edited spectra are shown in Figure 1. Patients had significantly lower GABA+ levels in the PCC ($p = 0.036$) and left hippocampus ($p = 0.039$) compared to healthy controls. However, no significant difference was observed in GABA+ concentrations in the mPFC ($p = 0.353$) between patients and controls (Table 2; Figure 2).

Correlation of GABA+ levels with cognitive function

In patients, significant positive correlations were observed between verbal memory and GABA+ concentrations in the left hippocampus (RAVLT-total immediate recall: $p = 0.01$; RAVLT-delayed recall: $p = 0.011$; RAVLT-total scores: $p = 0.016$). There was also a significant negative association between TMT scores and GABA+ concentrations in the PCC ($p = 0.016$) (Table 3; Figure 3). Although these correlations have differing mathematical polarity, they all follow the same direction with lower GABA levels corresponding to more abnormal cognitive scores. No significant correlations were found between GABA+ concentrations in the mPFC and any of the cognitive tests (Table 3).

Furthermore, a partial correlation analysis was used to rule out the effect of age. The results showed the same pattern as Pearson's correlation coefficients between GABA concentrations and cognitive performance. Verbal memory was significantly correlated with GABA+ concentrations in the left hippocampus (RAVLT-total immediate recall: $p = 0.003$; RAVLT-delayed recall: $p = 0.005$; RAVLT-total scores: $p = 0.007$). TMT scores were related to GABA+ concentrations in the PCC ($p = 0.004$). GABA+ concentrations in the mPFC were not correlated with cognitive tests in patients.

There were no significant associations between GABA+ levels in all the regions and cognitive tests in the control group (all p values > 0.05).

Discussion

Our study demonstrates that GABA+ concentrations were reduced in PCC and left hippocampus of patients with RRMS compared to age and gender-matched healthy controls. Significant correlations between GABA+ levels and cognitive performance were also observed in patients with RRMS, with lower GABA+ levels associated with worse cognitive performance in a number of domains. To our knowledge, this is the first study that reports reduced GABA+ concentrations in patients with RRMS *in vivo*. In addition, this study is also the first to demonstrate a relationship between GABA and cognitive function in RRMS patients.

GABA is primarily synthesised in GABAergic neurons from glutamate by glutamate decarboxylase (GAD) and catabolised via GABA-transaminase [33]. Thus, reduced GABA+ concentrations in our data are most likely related to GABAergic neuronal loss. Several preclinical [34, 35] and clinical (post-mortem tissue analysis) [36, 37] studies found decreased numbers of parvalbumin (PV)-positive GABAergic interneurons in MS brains. Additionally, Nicholas et al. detected loss of GABAergic interneurons in the middle temporal gyrus of autopsied multiple sclerosis brains [38], consistent with our findings. One study has shown a relationship between MRS GABA concentrations and genes coding for GAD, the main enzyme in GABA biosynthesis [39]. In addition, several studies found reduced levels of GAD65 and GAD67 in experimental autoimmune encephalomyelitis (EAE) rats [40], an animal model of multiple sclerosis, and decreased GAD67 in postmortem motor cortex from MS brains [36]. To conclude, our current finding of reduced GABA+ concentrations in patients with MS might also be due to a decline in the efficacy of production of GABA.

Our study furthermore revealed that abnormalities in the GABA system were present in the PCC and hippocampus of RRMS patients, supporting the suggestion that GABA might be a potential brain biomarker of neurodegeneration. These findings are in line with Cawley et al. [17], who examined right hippocampus GABA levels in secondary progressive multiple sclerosis patients. The reduction in GABA levels may be caused by dysfunctional GABA synthesis, or dysfunction in enzymes involved in glutamate-glutamine cycling. Consequently they may reflect abnormal plasticity, synaptic function and network activity in the hippocampus [41]. Further, studies of GABAergic drug treatment in EAE mice [42–44] also support the implication of dysfunctional GABAergic system in the pathogenesis of MS, hopefully forming the biological basis for future pharmacologic strategies.

Our results demonstrated decreased GABA+ levels in the PCC and hippocampal region. In contrast, Bhattacharyya et al. [18] found that there was no significant difference between sensorimotor GABA levels in controls and RRMS patients. These two studies differ in a number of key ways. Firstly, the two studies measured GABA in different brain regions. Secondly, the Bhattacharyya sample (13 RRMS patients vs. 10 healthy controls) was substantially smaller, diminishing statistical power. Thirdly, the MRS measurement volumes were also smaller (8 ml vs. ~18 ml), diminishing SNR of data and measurement precision. Finally, the inclusion of subjects taking GABAergic medications in Bhattacharyya study may also have impacted GABA levels. Our results also differ from a study recently done by Nantes et al. [45], which found relatively high sensorimotor GABA concentration among individuals with RRMS. Once again, different VOI was used, which may be the reason of obtaining different results. The current study did not observe any differences in GABA+ concentrations in the mPFC between patients and controls, suggesting that there may be some regional variation in decreased GABA levels in MS patients.

In this study, we also found significant correlations between decreased cognitive performance and lower GABA+ concentrations in the PCC and left hippocampal region in the RRMS group. Given that GABA is synthesised in inhibitory neurons [33], this suggests that loss and/or dysfunction of inhibitory neurons may be an important mechanism for cognitive impairment in multiple sclerosis. GABAergic interneurons provide recurrent inhibition to pyramidal neurons, an effect that is supposed to stabilise synchronised inhibitory neural network allowing for normal cognitive performance [46], therefore, loss or dysfunction of inhibitory neurons may result in cognitive disabilities. Reduced GABA levels observed in this study may imply deficits of GABA-mediated synaptic transmission, thus leading to altered inhibitory signalling within cortical circuits. Consequently, this may ultimately result in the loss or deterioration in cognitive function [46]. MS is an inflammatory, demyelinating neurodegenerative disease. In the context of chronic demyelination, decreased inhibitory innervation of cortical neurons may cause axonal loss by increasing the firing of demyelinated axons [36], while axonal injury and loss may contribute significantly to the cognitive decline in MS [1]. Additionally, demyelination and neuronal loss in the middle temporal (including the hippocampus) and cingulate cortices are of particular relevance to cognitive disturbance as these cortices are important for cortical binding and synchronisation and plasticity that impact cognitive functions [1]. Collectively, these studies provide enough evidence that GABAergic deficits in the PCC and hippocampus region can partially explain the cognitive impairment of patients with RRMS.

To our knowledge, no previous study has demonstrated the association between brain GABA levels and cognition in patients with RRMS. A recent MRS study found that higher sensorimotor GABA is associated with better SDMT scores in MS patients [45]. However, in this study, MS cohorts were heterogeneous (including primary progressive, secondary progressive and RRMS). The frequency and severity of cognitive impairment differ across MS phenotypes. Interestingly, Cawley et al. [17] reported no significant association between GABA levels in the right hippocampal or prefrontal cortex, and cognitive tests in progressive MS. This may be related to the fact that the patients described by Cawley et al. were secondary progressive multiple sclerosis compared to our population of RRMS, and that different neuropsychological tests were used. In addition, our results suggest dissociation between cognitive performance and GABA+ levels in the mPFC. Our results indicate regional specificity for the relation between GABA and cognitive function [47], and it is a complex relationship, in general and in MS specifically.

There are some limitations to our study. First, at 3T, the detected GABA+ signal using the MEGA-PRESS methodology contains a significant contribution (~50%) from co-edited macromolecules (MM). New methods for MM suppression are being developed to isolate GABA in the spectrum. However, these methods available have common practical limitations like being time-consuming and somewhat lacking the quality of the data. MM contamination is frequently accepted as a limitation of this most commonly applied approach [48]. Second, the VOIs were set relatively large due to the low intensity of the GABA+ signal, meaning the VOIs might have contained heterogeneous tissues, with small parts of neighbouring brain structures. Efforts should be made to use smaller VOIs to achieve reliable measurements of GABA concentrations in an acceptable acquisition time.

In conclusion, we found reduced GABA+ levels in the PCC and left hippocampus regions also in addition to significant correlations between GABA+ levels and cognitive impairment in patients with RRMS. These results support that GABAergic dysfunction may be implicated in the MS pathogenesis, suggesting a potential link between regional GABA levels and cognitive impairment in patients with RRMS.

Acknowledgments

Funding:

This study has received funding by the Shandong Provincial Key Research and Development Plan of China (2016ZDJS07A16) and the Shandong Provincial Natural Science Foundation of China (grant no. BS2015YY003) and the National Natural Science Foundation of China for Young Scholars (no. 81601479). This project was also supported by NIH grant R01 EB016089 and P41 EB015909.

Abbreviations

MS	Multiple sclerosis
RRMS	Relapsing-remitting multiple sclerosis
GABA	Gamma-aminobutyric acid
PCC	Posterior cingulate cortex

mPFC	Medial prefrontal cortex
MEGA-PRESS	Mescher-Garwood point resolved spectroscopy
FWHM	Full-width at half-maximum
EDSS	Expanded Disability Status Scale
EAE	Experimental autoimmune encephalomyelitis
GAD	Glutamic acid decarboxylase

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Key Points

- GABA levels may decrease in patients with RRMS.
- Lower GABA levels correlated with worse cognitive performance in patients with RRMS.
- Dysfunctional GABAergic neurotransmission may have a role in cognitive impairment in RRMS.

Figure 1.

The VOIs' position, corresponding segmentation data and representative spectra in a patient with RRMS using a MEGA-PRESS sequence. T1-weighted TFE images show the position of voxels in the posterior cingulate cortex (PCC) (a1, a2), medial prefrontal cortex (mPFC) (b1, b2) and left hippocampus (c1, c2) in the sagittal and coronal images. The corresponding results of brain segmentation are shown for PCC (a3), mPFC (b3) and left hippocampus (c3). Representative GABA+ spectra from PCC (a4), mPFC (b4) and left hippocampus (c4) are shown.

Figure 2.

Scatter plots show absolute GABA+ concentrations in institutional unit (IU) of (a), posterior cingulate cortex (PCC), (b), medial prefrontal cortex (mPFC), and (c), left hippocampus in MS patients and healthy controls (HCs). GABA+ concentrations of MS group are significantly decreased in the PCC ($p = 0.036$) (a) and left hippocampus ($p = 0.039$) (c). b: Shows that the mean GABA+ are lower in MS than in HCs in the mPFC, but no statistical difference is detected ($p = 0.353$). The within-scatter horizontal lines represent median values.

Figure 3.

Relationships between GABA+ concentrations and cognitive z scores in MS group in the left hippocampus and posterior cingulated cortex (PCC). Significant positive correlations were observed between GABA+ concentrations in the left hippocampus and verbal memory, with immediate recall (a), delayed recall (b) and RAVLT total scores (c). d: Shows that a significant negative correlation was observed between GABA+ concentrations in the PCC and TMT scores. RAVLT-I = Rey Auditory Verbal Learning Test (total immediate recall), RAVLT-II = Rey Auditory Verbal Learning Test (delayed recall), RAVLT= Rey Auditory Verbal Learning Test (total scores), TMT = Trail-making test.

Table 1

Demographics, clinical and cognitive scores of all subjects

	HCS	RRMS	Test Statistics	<i>p</i> -value
Number	26	28	-	-
Age (years)	37.6 ± 10.9	36.5 ± 10.0	<i>t</i> = 0.391	0.697
Number of females (%)	19 (73.1)	20 (71.4)	$\chi^2 = 0.018$	0.893
Education (years)	13.2 ± 4.6	11.9 ± 3.0	<i>t</i> = 1.257	0.216
Disease duration (years)	-	4.9 ± 3.5	-	-
Lesion number ^a	-	6 (0–45)	-	-
EDSS ^a	-	2 (0–4)	-	-
Cognitive Z-scores ^b				
RAVLT-I	-0.049 ± 0.146	-0.641 ± 0.141	<i>F</i> = 8.198	0.006
RAVLT-II	-0.091 ± 0.161	-0.869 ± 0.155	<i>F</i> = 11.908	0.001
RAVLT	-0.1 ± 0.154	-0.798 ± 0.149	<i>F</i> = 10.44	0.002
RCFT	-0.031 ± 0.168	-0.374 ± 0.162	<i>F</i> = 2.089	0.155
Stroop	0.083 ± 0.144	0.194 ± 0.138	<i>F</i> = 0.302	0.585
SDMT	-0.047 ± 0.11	-0.493 ± 0.106	<i>F</i> = 8.18	0.006
TMT	-0.032 ± 0.149	0.644 ± 0.143	<i>F</i> = 10.73	0.002

Data are presented as mean ± SD.

^aIndicates median and range.^bCognitive scores for healthy controls and patients corrected for age, gender and educational level.

HCS = healthy controls, RRMS = relapsing-remitting multiple sclerosis, EDSS = Expanded Disability Status Scale, RAVLT-I = Rey Auditory Verbal Learning Test (total immediate recall), RAVLT-II = Rey Auditory Verbal Learning Test (delayed recall), RAVLT = Rey Auditory Verbal Learning Test (total score), RCFT = Rey-Osterich complex figure test, Stroop = Stroop color word interference test, SDMT = Symbol-Digit Modalities Test, TMT = Trail-making test.

Table 2

MRS and segmentation data of patients and controls

	HCs	RRMS	Test Statistic	<i>p</i> -value
PCC				
GABA+ level (IU)	1.14 ± 0.13	1.06 ± 0.15	<i>F</i> = 4.655	0.036
GABA+fitting errors (%)	6.21 ± 0.99	6.57 ± 0.87	<i>t</i> = -1.426	0.16
FWHM (Hz)	7.23 ± 0.51	7.00 ± 0.67	<i>t</i> = 1.416	0.163
Volume of GM (cm ³)	10.13 ± 0.61	10.13 ± 0.66	<i>t</i> = 0.05	0.961
GM/(GM + WM) (%)	60.49 ± 3.92	60.53 ± 4.44	<i>t</i> = -0.034	0.973
mPFC				
GABA+ level (IU)	1.10 ± 0.27	1.04 ± 0.17	<i>F</i> = 0.88	0.353
GABA+fitting errors (%)	8.46 ± 1.35	8.77 ± 2.11	<i>t</i> = -0.632	0.53
FWHM (Hz)	10.38 ± 1.36	10.04 ± 1.80	<i>t</i> = 0.801	0.427
Volume of GM (cm ³)	10.36 ± 0.49	10.35 ± 0.53	<i>t</i> = 0.106	0.916
GM/(GM + WM) (%)	64.78 ± 3.25	64.48 ± 3.37	<i>t</i> = 0.331	0.742
Left hippocampus				
GABA+ level (IU)	1.33 ± 0.27	1.18 ± 0.23	<i>F</i> = 4.504	0.039
GABA+fitting errors (%)	6.95 ± 1.41	7.39 ± 2.07	<i>t</i> = -0.915	0.365
FWHM (Hz)	12.46 ± 1.45	12.07 ± 1.63	<i>t</i> = 0.926	0.359
Volume of GM (cm ³)	8.14 ± 0.57	8.25 ± 0.48	<i>t</i> = -0.755	0.453
GM/(GM + WM) (%)	52.87 ± 3.75	53.78 ± 3.34	<i>t</i> = -0.942	0.351

Data are presented as mean ± SD.

HCs = healthy controls, RRMS = relapsing-remitting multiple sclerosis, PCC = posterior cingulate cortex, mPFC = middle pre-frontal cortex, GABA+ = GABA plus co-edited macromolecules, FWHM = full-width at half-maximum, GM = gray matter, WM = white matter.

Table 3

Correlations between GABA+ concentrations and cognitive scores in MS patients

	PCC		mPFC		Left hippocampus	
	<i>r</i>	<i>p-value</i>	<i>r</i>	<i>p-value</i>	<i>r</i>	<i>p-value</i>
RAVLT-I	-0.014	0.942	-0.096	0.634	0.477	0.010
RAVLT-II	-0.04	0.84	-0.191	0.34	0.471	0.011
RAVLT	-0.025	0.9	-0.142	0.481	0.451	0.016
RCFT	-0.135	0.493	-0.106	0.599	-0.231	0.237
Stroop	-0.308	0.111	0.073	0.716	-0.078	0.693
SDMT	0.037	0.85	-0.062	0.76	-0.173	0.378
TMT	-0.452	0.016	-0.185	0.355	0.049	0.806

GABA+ = GABA plus co-edited macromolecules, PCC = posterior cingulate cortex, mPFC = middle pre-frontal cortex, RAVLT-I = Rey Auditory Verbal Learning Test (total immediate recall), RAVLT-II = Rey Auditory Verbal Learning Test (delayed recall), RAVLT = Rey Auditory Verbal Learning Test (total scores), RCFT = Rey-Osterich complex figure test, Stroop = Stroop colour word interference test, SDMT = Symbol-Digit Modalities Test, TMT = Trail-making test.