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# Sensorimotor Cortex Gamma-Aminobutyric Acid Concentration Correlates with Impaired Performance in Patients with MS

P.K. Bhattacharyya, M.D. Phillips, L.A. Stone, R.A. Bermel, and M.J. Lowe



## ABSTRACT

**BACKGROUND AND PURPOSE:** Abnormalities in GABA concentration [GABA] have been associated with several neuropsychiatric disorders, and research has suggested that GABA may play a role in sensorimotor cortex function. We sought to determine whether identifying a change in [GABA] within the sensorimotor cortex of patients with MS has any effect on motor function and would provide information about the adaptive/compensatory mechanisms involved in the attempt to maintain motor function during disease progression.

**MATERIALS AND METHODS:** In 19 healthy controls and 30 patients with MS, we assessed task performance with the MS Functional Composite scale and its components (T25FW test, 9HPT, and PASAT). With in vivo MR spectroscopy, we measured [GABA] in the sensorimotor cortex and determined correlations between [GABA] and task performance. We also assessed the association between [GABA] and cortical activation volume after a bilateral finger-tapping task.

**RESULTS:** [GABA] was inversely correlated with 9HPT scores in patients with MS, indicating a worsening of performance with increased [GABA]. No significant correlation was observed between [GABA] and T25FW or PASAT scores. [GABA] was directly correlated with primary motor cortex activation volume after the finger-tapping task in patients with MS.

**CONCLUSIONS:** These results suggest that cortical [GABA] may be a marker of function and reorganization/adaptation of cortical gray matter in MS.

**ABBREVIATIONS:** 9HPT = Nine-Hole Peg Test; GABA =  $\gamma$ -aminobutyric acid; GM = gray matter; PASAT = Paced Auditory Serial Addition Test; PRESS = point-resolved spectroscopy sequence; T25FW = Timed 25-Foot Walk Test

Spectroscopic measurement of GABA has garnered attention in recent years because of the role of GABA as a major inhibitory transmitter in the human brain. Abnormalities in GABA concentration [GABA] have been associated with several neuropsychiatric disorders,<sup>1</sup> and recent reports have suggested that GABA may

play a role in sensorimotor cortex function in both healthy and disease states.<sup>2-9</sup> In particular, GABA may be involved in brain plasticity, cortical adaptation, and reorganization in neurodegenerative disease processes. Reduction in GABA inhibition has been reported to facilitate long-term potentiation-like activity in the motor cortex.<sup>3,4</sup> GABAergic inhibition has been identified as one of the mechanisms operating in use-dependent plasticity in the intact human motor cortex, which suggests similarities between the mechanisms underlying this form of plasticity and long-term potentiation.<sup>2</sup> The GABA agonist lorazepam is associated with suppression of profound reorganization in the somatosensory cortex, as demonstrated with motor-learning paradigms.<sup>8</sup> [GABA] reduction in the sensorimotor cortex of healthy controls during motor learning has been reported<sup>5</sup>; and decreases in [GABA] in the sensorimotor cortex have been observed in patients with focal dystonia.<sup>6</sup> Reduction of [GABA] in the sensorimotor cortex induced by acute deafferentation has also been reported.<sup>7</sup> A recent study reported a positive correlation between the decrease in primary motor cortex (M1) [GABA] and the degree of motor learning as well as the motor learning-induced fMRI signal change within the M1.<sup>9</sup>

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MS frequently produces significant motor impairment in affected patients. Damage to both GM and WM components of the motor system has been reported in patients with MS.<sup>10,11</sup> Functional imaging studies have suggested that adaptive and/or compensatory mechanisms are involved in the attempt to maintain motor function during disease progression.<sup>10,12-14</sup> These studies generally have demonstrated an increase in the extent of functional cortical activation in patients with MS versus controls during performance of specific tasks; this increased brain activation has been interpreted as cortical reorganization and/or adaptation. Given the role of GABA in other disease processes, we hypothesized that identifying a change in [GABA] within the sensorimotor cortex of patients with MS would provide us with additional information about the ongoing disease process and these compensatory mechanisms.

In this study, we measured the sensorimotor cortex [GABA] in healthy controls and in patients with MS with MR spectroscopy. These [GABA] values were correlated with the MS Functional Composite<sup>15</sup> scores and with scores of the individual components of the scale involving ambulatory, arm function, and cognitive tasks. Additionally, to investigate the potential role of GABA in cortical reorganization, we assessed the association between [GABA] and cortical activation volume following a bilateral finger-tapping task.

## MATERIALS AND METHODS

Nineteen healthy controls and 30 patients with relapsing-remitting MS participated in this study. The patients had their last doses of steroids at least 8 weeks before the MR imaging and were clinically stable, with no changes in the Expanded Disability Status Scale for at least 8 weeks before imaging. From these participants, datasets for 8 controls and 13 patients were discarded because of unacceptable subject motion.<sup>16,17</sup> GABA signal was either too low or inconclusive in the datasets for 1 control and 4 patients (the spectra were either too noisy to fit with the Advanced Method for Accurate, Robust, and Efficient Spectral fitting,<sup>18</sup> or had other comparable peaks present in the edited spectra, possibly due to poor editing). Data from the remaining 10 healthy controls (8 women, 2 men; mean age = 38.4 ± 13.6 years; mean MS Functional Composite = 0.408 ± 0.445) and 13 patients with MS (10 women, 3 men; mean age = 47.2 ± 8.3 years; disease duration = 9.5 ± 7.0 years; mean Expanded Disability Status Scale score = 3.15 ± 2.30; mean MS Functional Composite = 0.212 ± 0.559) were included in this study. Sixteen of a total 96 time points were discarded in 1 acceptable dataset because of motion, while no other dataset had to be discarded among the acceptable studies. The local institutional review board approved the study procedures, and all patients and controls provided informed consent.

### Behavioral Study

The behavioral study consisted of MS Functional Composite testing with 3 components: 1) the T25FW test, a measure of ambulation in which the time required for the subject to walk 25 feet back and forth is recorded; 2) the 9HPT, a measure of arm function and fine-motor coordination, in which the time taken by the subject to pick up and place 9 dowels in 9 holes is recorded<sup>19</sup>; and 3) the PASAT, a measure of cognition in which the subject is presented

with a series of 60 numbers spaced 2 seconds apart and asked to add the 2 most recent numbers and the number of correct responses is recorded.<sup>20</sup> The z score for each component and the total MS Functional Composite score for each subject were calculated with the methods outlined by the National Multiple Sclerosis Society Clinical Outcomes Assessment Task Force.<sup>15,21,22</sup>

### MR Imaging Study

MR images were obtained on a 3T Trio scanner (Siemens, Erlangen, Germany). A circularly polarized head coil was used. All patients and healthy volunteers were scanned with a sequence based on the MEGA-PRESS sequence that was designed by Mescher et al.<sup>23</sup>

To identify motion-corrupted data for all controls and the first 25 patients with MS, we used water signal-based interleaved navigator pulses,<sup>17</sup> a methodology that has been identified as an effective way to discard only the portion of data from a scan that is motion-corrupted and to therefore reduce possible misinterpretation of the edited spectra. For the last 5 patients with MS, we used an alternative method of motion detection. This method identified motion by collecting data in weak water-suppression mode to detect residual water-signal fluctuation.<sup>16</sup> This change in the motion-identification method was implemented to improve the signal and ease of acquisition and has similar efficacy in detecting motion compared with the first method. Because the main MEGA-PRESS module used in the 2 methods is the same, a change in motion-detection method does not influence the measured GABA concentration. This was verified by scanning phantoms containing GABA of a known concentration. Three percent or more change in water signal is indicative of unacceptable motion in both methods.<sup>16,17</sup>

The complete protocol consisted of the following scans, as discussed in our previous work<sup>24</sup>:

- 1) A localizer scan.
- 2) Gradient-echo field mapping.
- 3) A whole-brain T1-weighted magnetization-prepared rapid acquisition of gradient echo scan with the following parameters: 144 axial sections; thickness = 1 mm; FOV = 25.6 × 25.6 cm; TR = 1900 ms; TE = 1.89 ms; flip angle = 8°; 256 × 128 matrix; readout bandwidth = 125 kHz; and scan time = 8 minutes 5 seconds.
- 4) An fMRI scan: The gradient-echo echo-planar fMRI scan parameters were as follows: TR = 2000 ms; TE = 30 ms; flip angle = 90°; number of transverse sections = 32; and section thickness = 4 mm without any intersection gap. Subjects performed self-paced bilateral finger tapping (index finger simultaneously in opposition to the thumb on each hand) in blocks of interleaved 32-second ON and 32-second OFF patterns. The voxel at the motor cortex was selected by using the real-time fMRI Student *t* statistic activation map-generation program Neuro 3D (Siemens). A single voxel (2 × 2 × 2 cm<sup>3</sup>) centered at the area of maximum activation in the precentral gyrus of the right hemisphere was selected for the spectroscopy scans from pixels with *t* ≥ 4.0 (*P* < .001).

- 5) A MEGA-PRESS-based GABA editing scan with a water signal-based interleaved navigator scan<sup>17</sup> by using the following parameters: voxel = 2 × 2 × 2 cm<sup>3</sup>; TR = 2700 ms; TE = 68 ms;

number of excitations = 96; flip angle = 90°; unsuppressed water-excitation flip angle = 20°; edit pulse frequency = 1.90 ppm (the other 180° pulse was placed at 1.50 ppm to minimize macromolecule contamination<sup>25</sup>); edit pulse width ~ 44 Hz (duration = 20 ms); and scanning time = 8 minutes 39 seconds. The navigator-based sequence was replaced with MEGA-PRESS in weak water-suppression mode for the last 5 patients.<sup>16</sup>

6) A metabolite nulling scan with an 180° inversion pulse added to the sequence used in scan 5 with a TI of 650 ms.<sup>24</sup> Even though pulsing at 1.90 and 1.50 ppm minimizes macromolecule contamination, this scan was added to ensure minimization of any residual macromolecule contamination resulting from shot-to-shot B0 variation.<sup>26</sup>

7) A PRESS scan with TR = 2700 ms and number of excitations = 48.

8) A PRESS scan with TR = 2700 ms, number of excitations = 1, and no water suppression.

9) Repeat of the localizer scan.

A bite-bar was used during all scans to reduce head motion.

The MR spectroscopy data were analyzed with the jMRUI software package (<http://www.mrui.uab.es/mrui>)<sup>27</sup>; we described the steps of this data analysis in detail in a previous study.<sup>24</sup> In brief, the data analysis consisted of the following: 1) discarding the first 4 measurements from each scan to ensure steady-state magnetization and identifying and discarding motion-corrupted data on the basis of fluctuation of the unsuppressed navigator water/residual water signal; 2) performing zero-order phase correction; 3) performing frequency-shift correction of the individual subspectra by using residual water as a reference; 4) summing the phase- and frequency-corrected subspectra; 5) performing residual water suppression with a Hankel-Lanczos singular-value decomposition filter<sup>28</sup>; 6) performing apodization with a 5-Hz Gaussian filter; and 7) performing zero-filling. Finally, the OFF-resonance spectrum was subtracted from the ON-resonance spectrum to obtain the final edited spectrum.

Next, as we previously described,<sup>24</sup> the GM, WM, and CSF contributions to the voxel composition were determined with the fMRI of the Brain Automated Segmentation Tool (<http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FAST>) algorithm<sup>29</sup> of the fMRI of the Brain Software Library (<http://www.fmrib.ox.ac.uk/analysis/techrep/tr04ss2/tr04ss2/node19.html>)<sup>30</sup>; the anatomic 3D magnetization-prepared rapid acquisition of gradient echo scan was used as the base image, and a mask was applied at the voxel location. The fraction volumes of gray matter, white matter, and CSF ( $f_{GM\_vol}$ ,  $f_{WM\_vol}$ , and  $f_{vol\_CSF}$ ) were used to perform absolute quantification of the GABA level.

The absolute GABA level was obtained following a 2-step process as described in our previous study<sup>24</sup>:

1) The [GABA]/[Cr] ratio was obtained in the first step from scans 5 and 6 by using the equation:

$$\frac{[GABA]}{[Cr]} = \frac{(G^* - M)}{I_{Cr} \times EE},$$

where  $G^*$  is the area under the 3.01-ppm peak in the edited spectrum,  $M$  is the area under the 2.99-ppm residual macromolecule peak in the metabolite-nulled spectrum after correcting for the relaxation effects,  $I_{Cr}$  is the area under the 3.93-ppm peak (meth-

ylene Cr) in the OFF-resonance spectrum, and  $EE$  is the editing factor. All areas were measured by using the Advanced Method for Accurate, Robust, and Efficient Spectral fitting algorithm.<sup>17</sup> The editing factor  $EE$  was calculated following the method used by Terpstra et al<sup>26</sup> by comparing the unmodulated GABA relative to the glycine signal from a PRESS scan (TR = 2700 ms, TE = ms) with that from a MEGA-PRESS scan of the same voxel of a phantom containing GABA and glycine. Terpstra et al<sup>26</sup> had assumed an identical T2 of Cr methyl and C<sup>4</sup>H GABA resonances, and T2 of Cr methyl and methylene resonances were reported at the occipital lobe by Mlynárik et al<sup>31</sup> at 3T. Assuming the T2 of Cr resonances in occipital and motor areas to be similar, we calculated the difference in T2 relaxation effect between Cr methyl and methylene at TE = 68 ms to be negligible, and the T2 of the Cr methylene and of the C<sup>4</sup>H GABA resonances was assumed to be identical in this study. In addition, the potential difference in the T1 relaxation effect at TR = 2700 ms was assumed to be negligible in this study, in line with the assumption of comparable T1 of all metabolites<sup>23</sup> and our observation of similar T1 of GABA and Cr from phantom scans. In this study, we used the methylene resonance of Cr instead of the methyl resonance because the latter can introduce a systematic error in [GABA]/[Cr] measurement.<sup>32</sup>

2) Next, the creatine concentration, [Cr], was determined from scans 7 and 8 by using the following equation as in Gasparovic et al<sup>33</sup>:

$$[Cr] = I_{Cr} \times \frac{(f_{GM} \times R_{H2O\_GM} + f_{WM} \times R_{H2O\_WM} + f_{CSF} \times R_{H2O\_CSF})}{S_{H2O\_obs} (1 - f_{CSF}) \times R_{Cr}} \times \frac{2}{\#H_{Cr}} \times H_2O,$$

where  $I_{Cr}$  is the area under the 3.93-ppm peak in scan 7, and  $f_{GM}$ ,  $f_{WM}$ , and  $f_{CSF}$  are the fractions of GM, WM, and CSF water respectively.  $R_{H2O\_GM}$ ,  $R_{H2O\_WM}$ , and  $R_{H2O\_CSF}$  are the relaxation attenuation factors for water in GM, WM, and CSF respectively;  $S_{H2O\_obs}$  is the area under the unsuppressed water peak in scan 8;  $R_{Cr}$  is the relaxation attenuation factor for Cr methylene resonance;  $\#H_{Cr}$  ( $n = 2$ ) is the number of protons in Cr methylene; and  $[H_2O]$  (55 mol/L) is the concentration of pure water.  $f_{GM}$ ,  $f_{WM}$ , and  $f_{CSF}$  were calculated by using  $f_{GM\_vol}$ ,  $f_{WM\_vol}$ , and  $f_{vol\_CSF}$  as in Bhattacharyya et al<sup>24</sup> and Gasparovic et al.<sup>33</sup>

Finally, the GABA concentration was determined by taking the product of the [GABA]/[Cr] ratio and [Cr].

Because the water content in an MS lesion is  $6.3 \pm 0.3\%$  higher than that in normal-appearing WM<sup>34</sup> and the water content in normal-appearing WM is approximately 2.2% higher than that in normal WM,<sup>34</sup> we made the necessary corrections for calculating [GABA] in patients with MS. It should be pointed out that the WM lesion content within spectroscopy voxels as determined from the T1-weighted image was only approximately 0.2%.

To account for the effect of voxel composition in patients with MS, we estimated [GABA] in GM and WM. It has recently been shown, with linear regression analysis, that [GABA] values within GM and WM in the sensorimotor region are  $2.87 \pm 0.61$  and  $0.33 \pm 0.11$  mmol/L, respectively, in healthy controls, which results in a GM/WM [GABA] ratio of  $8.70 \pm 3.44$ .<sup>24</sup> Because

[GABA] in the cortical GM may vary among patients depending on the severity of disease, we determined that it was not feasible to perform linear regression analysis on patient data. To estimate the GM/WM [GABA] in patients, we therefore assumed that the ratio of GM and WM [GABA] was similar in patients with MS and controls. In the absence of any a priori knowledge, we performed the analysis several times by using different GM and WM [GABA] ratios, ranging from 5 to 11.

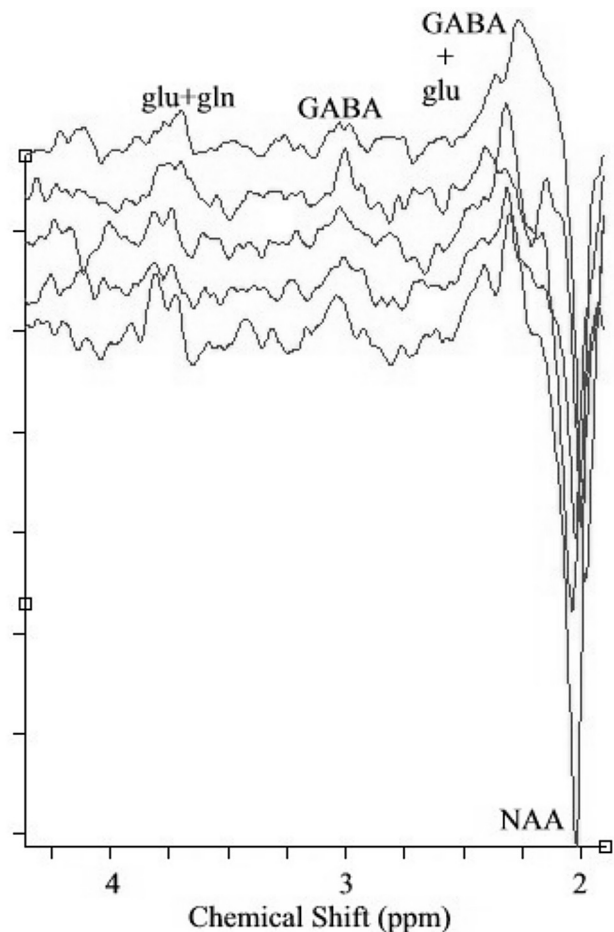
We analyzed the fMRI data to look for a correlation between activation volume and [GABA]. The first 4 volumes from the fMRI time-series were discarded. The remaining data were spatially filtered by using a 64-point radially symmetric 2D Hamming filter in the Fourier domain and were then retrospectively motion-corrected by using Analysis of Functional NeuroImages software (AFNI; <http://dbic.dartmouth.edu/wiki/index.php/AFNI>).<sup>35</sup> Data were analyzed for activation by least-squares fitting the time-series for each pixel to a boxcar reference function plus a slope.<sup>36</sup> Student *t* maps and a magnetization-prepared rapid acquisition of gradient echo scan for each subject were transformed into the standard stereotaxic space defined by Talairach and Tournoux<sup>37</sup> by using AFNI.<sup>35</sup> ROI analysis was performed by using the Human Motor Area Template (<http://lrnlab.org/>), a set of ROI masks defining the M1, primary sensory cortex, dorsal premotor cortex, ventral premotor cortex, and supplemental motor area in Talairach space.<sup>38</sup> The number of activated voxels (Student *t* > 3.5, 1-sided, uncorrected  $P < 3 \times 10^{-4}$ ) within the Human Motor Area Template ROI mask corresponding to M1 was determined. The right hemisphere of the brain was chosen for this analysis because the spectroscopy voxel was selected from within the right hemisphere for each subject.

## RESULTS

Figure 1 shows spectra from 5 subjects (all patients) obtained by using a MEGA-PRESS sequence. Table 1 shows the [GABA]/[Cr] ratio, [Cr], and [GABA] in a  $20 \times 20 \times 20 \text{ mm}^3$  voxel in the sensorimotor region of the healthy controls and patients with MS. [GABA]/[Cr] and [GABA] were not significantly different between controls and patients ( $P = .10$  and  $0.15$ , respectively).

The calculated correlation coefficients between MS Functional Composite scores (and the components of the MS Functional Composite) and [GABA] are shown in Table 2. We observed a very strong inverse correlation between [GABA] and the 9HPT in patients with MS (Fig 2). Because the spectroscopy voxels were always selected from the right hemisphere, we repeated the same analysis by using 9HPT data for the contralateral (left) hand only. We also repeated the analysis by using only the cortical GM [GABA], for which we used the range of GM [GABA], as mentioned earlier. In all cases, significant inverse correlation between 9HPT scores and [GABA] was observed. Most interesting, the control subjects' data did not show any such correlation ( $P = .78$ ). The [GABA]/[Cr] ratio did not significantly correlate with the MS Functional Composite or its components for either controls or patients. Combined plots of [GABA] versus 9HPT are shown in On-line Fig 1.

The total number of M1 voxels was  $497 \pm 50$  and  $498 \pm 64$  in controls and patients, respectively. The number of activated voxels in the controls ( $124 \pm 92$ ) was not significantly different from



**FIG 1.** GABA-edited spectra from 5 subjects (all patients) obtained by using MEGA-PRESS sequence. NAA and coedited glutamate (glu) and glutamine (gln) can be seen in the spectra in addition to GABA.

**Table 1: GABA and Cr concentrations in healthy controls and patients with MS<sup>a</sup>**

	Voxel Composition			[GABA]/[Cr]	[Cr] (mM)	[GABA] (mM)
	% GM	% WM	% CSF			
Controls (n = 10)	37 ± 7	52 ± 12	11 ± 8	0.15 ± 0.05	9.22 ± 0.85	1.43 ± 0.48
Patients (n = 13)	34 ± 7	49 ± 10	16 ± 5	0.19 ± 0.06	9.43 ± 1.43	1.71 ± 0.42

<sup>a</sup> Values are means.

that in patients with MS ( $176 \pm 63$ ). The trend of our data suggests a significant correlation between the number of activated voxels (ie, activation volume) and [GABA] in the patients with MS ( $P = .05$ ; Fig 3). No significant correlation was seen between activation volume and [GABA] in the healthy controls ( $P = .81$ ). The [GABA]/[Cr] ratio did not have any significant correlation with primary cortex activation volume in either controls ( $P = .90$ ) or patients ( $P = .16$ ). Combined plots of [GABA] versus activation volume are shown in On-line Fig 2.

## DISCUSSION

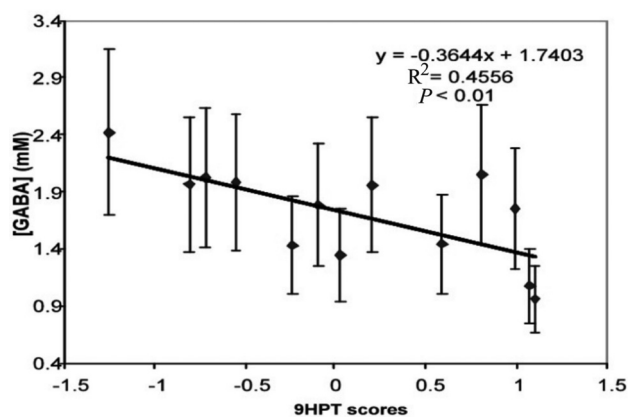
In this study, we found that [GABA] was inversely correlated with 9HPT scores in patients with MS, indicating a worsening of performance with increases in [GABA]. The [GABA] value was also directly correlated with primary motor cortex-activation volume after a bilateral finger-tapping task in patients with MS.

**Table 2: Correlation coefficients of MSFC and components with GABA concentration and the ratio of GABA and Cr concentration**

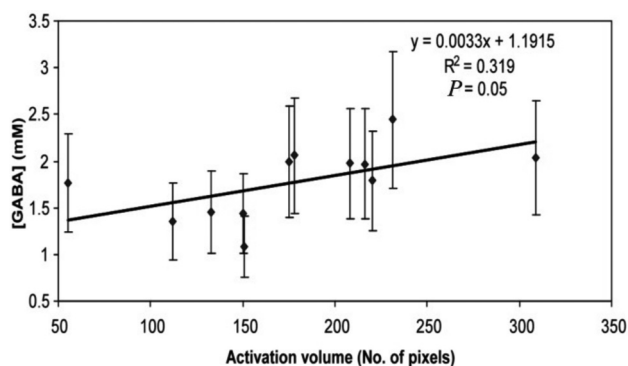
	GABA MSFC	GABA/Cr MSFC	GABA 9HPT	GABA/Cr 9HPT	GABA T25FW	GABA/Cr T25FW	GABA PASAT	GABA/Cr PASAT
Controls (n = 10)	-0.2233, P = .54	-0.1728, P = .63	0.1035, P = .78	0.1824, P = .61	-0.1680, P = .64	-0.0133, P = .97	-0.3341, P = .35	-0.3124, P = .38
Patients (n = 13)	-0.3324, P = .27	-0.2052, P = .50	-0.6750, <sup>a</sup> P = .01	-0.4807, P = .1	-0.3865, P = .19	-0.4155, P = .16	0.0504, P = .87	0.1161, P = .71

Note:—MSFC indicates Multiple Sclerosis Functional Composite.

<sup>a</sup>Statistically significant.



**FIG 2.** Inverse correlation of sensorimotor  $\gamma$ -aminobutyric acid concentration and Nine-Hole Peg Test scores in patients with multiple sclerosis. A fixed error bar of 30% is used for [GABA] in the plot.



**FIG 3.** Correlation of sensorimotor  $\gamma$ -aminobutyric acid concentration and fMRI activation volume in patients with multiple sclerosis. A fixed error bar of 30% is used for [GABA] in the plot.

Recent histopathologic observations have suggested that GABA receptor levels may be reduced in the motor cortex of patients with MS,<sup>39,40</sup> whereas our results suggest that [GABA] is increased within the motor cortex in patients who demonstrate poorer motor performance. The reasons for these different findings are unclear. It is possible that these varying results may reflect differences in the patient populations evaluated; the pathologic studies included predominantly patients with late end-stage chronic MS, whereas we included patients with relapsing-remitting MS.

The 9HPT is a clinical measure that probes dominant and nondominant hand and finger coordination and dexterity. Thus, this test more specifically addresses motor function in a manner similar to finger tapping, which was used for GABA spectroscopy voxel selection. In our study, no significant correlation was found between [GABA] and the T25FW or PASAT components of the MS Functional Composite in patients with MS. These results, in

conjunction with the inverse correlation between [GABA] and the 9HPT in study patients, suggest that [GABA] measurements within a given cortical region relate directly to the expected function of the underlying parenchyma rather than reflecting a generalized effect of global disease progression.

While several investigators have studied the relationship between GABA and motor plasticity in healthy controls,<sup>2,5,9,41</sup> the work has focused on tasks involving motor learning. In this study, we adopted a task that does not involve motor learning. Our observation of lack of any correlation between sensorimotor [GABA] and M1 activation volume in healthy controls is in line with a previous observation of no sensorimotor [GABA] modulation during unlearnable nonrepetitive tasks.<sup>5</sup> Cortical reorganization or adaptation has been found to take place in patients with MS as a compensatory mechanism for functioning.<sup>10,12-14</sup> This reorganization/adaptation is manifested by increased activation, as measured by fMRI, involving a broader range of cortical regions in these patients versus controls. Reddy et al<sup>10</sup> reported increased activation in the ipsilateral sensorimotor cortex with simple hand movements in patients with MS. In another study, a distinct pattern of cortical activation from disability and brain injury in MS was observed.<sup>13</sup> The degree of adaptive cortical functional change has also been shown to be correlated with the extent of brain injury in patients with nondisabling MS.<sup>14</sup> The underlying mechanism responsible for this cortical reorganization or adaptation is still unknown, and a cortical marker of adaptation has not previously been explored in great detail. Our observation of increased cortical [GABA] with increased motor activation suggests that GABA plays a role in this cortical adaptation process.

Although the inclusion criteria for datasets in our study reduced the final sample size, the increased reliability of GABA measurements was considerable; additionally, the inverse correlation between [GABA] and 9HPT scores and the correlation between [GABA] and the number of activated pixels were statistically significant.

Potential differences in self-paced finger tapping between individuals is a limitation of this study. Because motor learning has been reported to the sensorimotor cortex GABA levels,<sup>5</sup> we chose self-paced finger tapping as the task for this study instead of having the subjects follow a specific pattern. Our experience with this in past studies<sup>42,43</sup> is that similar paradigms have sufficiently light attention demands so that patients with MS can easily perform them with little training and the variability in performance is minimal.

Also some of the medications prescribed to the patients may have some effect on GABA levels. In addition, modulation of the occipital cortex GABA level during the menstrual period has been reported in literature<sup>44</sup> and may have some effect in our sensorimotor GABA measurement. However, function-specific correla-

tions suggest that the effect we are observing primarily arises from the MS disease process.

In addition, heterogeneity in subject population could potentially dilute some of the changes in the components in the MS Functional Composite. This is another potential limitation of the study. We have reported an interesting observation that suggests the role of the cortical GABA level in MS. At this stage, we are not in a position to comment on the mechanism of this observation at the cellular level or the implication of this in GABAergic drug medications. Another limitation of this study is the small subject population. The study was not powered to find changes in T25FW or PASAT. Further studies are needed to explore this, and we are pursuing that as well. However, our observation with the current sample size strongly suggests that sensorimotor [GABA] in MS is inversely related to the 9HPT and not correlated with the T25FW or PASAT.

A note on multiple comparisons is in order. Although multiple hypotheses were tested in the work reported here, we think that a Bonferroni correction is not warranted, due to the fact that we had a priori expectations for each of the tests. Our principal hypothesis was that a strong significant inverse correlation was observed between [GABA] and the 9HPT in patients. Because [GABA] was measured at the sensorimotor cortex and the 9HPT directly addresses a function that involves the sensorimotor cortex, we had a priori expectations that these would correlate. Similarly, because [GABA] was measured at the sensorimotor cortex and like the 9HPT, finger tapping directly addresses a function that involves the sensorimotor cortex, we had an a priori expectation that the volume of activation in the primary motor cortex would be correlated with [GABA]. On application of a strict multiple-comparison correction, the volume correlation loses significance. However, given the a priori nature of the comparison and the exploratory nature of the study, we think that such a correction is not warranted.

## CONCLUSIONS

We found that [GABA] in the region of the sensorimotor cortex associated with hand activity in patients with MS was inversely correlated with performance on the 9HPT, a task that involves hand coordination. Additionally, [GABA] had no significant correlation with tasks not involving hand activity and the associated brain regions, suggesting that these results are functionally and anatomically specific. We also observed that [GABA] was directly correlated with activation volume in the primary motor cortex after self-paced bilateral finger tapping. No such correlations were observed between [GABA] and 9HPT performance or primary motor cortex activation in healthy controls. These results suggest that cortical [GABA] may play a role in and be a potential marker of function and reorganization/adaptation of cortical GM in patients with MS.

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