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Original Article

GABA-induced motor improvement following acute cerebral infarction

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Abstract: γ-Aminobutyric acid (GABA) plays a key role in motor learning. In the aftermath of stroke, we monitored GABA+ content of primary motor cortex by magnetic resonance spectroscopy (MRS), assessing its relation to functional motor recovery following a standardized 4-week program of rehabilitation. The cohort included 20 patients, each experiencing stroke within 2 weeks of symptom onset. Twenty age-matched healthy subjects were also recruited as controls. GABA+ levels were determined at baseline and following rehabilitation, performed only once in sex- and age-matched control subjects. Motor functions were then measured via Fugl-Meyer Assessment (FMA). Processing of MRS data was driven by open-source Gannet software. Because GABA, macromolecules, and homocarnosine jointly contribute to MEscher-Garwood Point RESolved Spectroscopy (MEGA-PRESS) signals, the designation GABA+ (rather than GABA) was applied. Baseline GABA+/creatine (Cr) ratios proved significantly lower in patients with strokes than in control subjects (P<0.05). Following the 4-week rehabilitative regimen, significant improvement in FMA indices was evident across the test group. FMA scores and GABA+/Cr ratios correlated significantly at baseline, the GABA+/Cr ratio displaying a significant association with motor function (P=0.025). In the setting of acute stroke, GABA+/Cr ratios of primary motor cortex fell significantly below levels found in healthy subjects. The observed association between GABA+/Cr ratio and motor recovery underscores the utility of MRS-measured GABA as a key motor recuperative biomarker.

Keywords: Stroke, GABA, magnetic resonance spectroscopy, rehabilitation

Introduction

Stroke remains the leading cause of neurological disability across the globe. Its reported incidence is ~17 million annually [1], leaving 5 million people with lingering disabilities [2]. Although treatments administered during acute phases of stroke have recently improved, therapies that aid in long-term recuperation have stalled. A key element of physical function after stroke is the recovery of motor skills through physiotherapy, but required interventions may be costly, limited, and confer negligible benefits to residual cortex [3]. Nearly one-half of all patients with strokes will become disabled and dependent on helpers for daily life activities [4]. Unfortunately, the molecular mechanisms of stroke recovery are as yet unclear. We believe that a better understanding of this process, addressing related neurobiochemical changes, may facilitate the development of effective interventions for stroke.

Rehabilitation from stroke relies on neuronal re-learning and brain plasticity [5], both modulated by GABAergic inhibition [6]. The pivotal role of GABAergic disinhibition in promoting primary motor cortex (M1) plasticity has been shown through two-photon *in vivo* imaging of M1 in mouse models [7]. In this context, learning of motor skills led to loss of axonal boutons on somatostatin-expressing inhibitory neurons [7]. Compared with healthy individuals, reduced GABA+ levels in M1 have also been reported during chronic stroke recovery [8]. Such reductions correspond with motor improvement after therapeutic intervention [8]. Optogenetically manipulating activity in inhibitory neuronal populations during learning disrupts these structural dendritic changes, subsequently influenc-

ing motor performance. It has been suggested that M1 disinhibition following transcranial magnetic stimulation (TMS) is controlled by the GABAergic system [9]. These studies collectively highlight the relevance of GABAergic inhibition in post-stroke recovery, although all patients investigated were in chronic phases. GABA fluctuations in patients whose motor functions improved after acute stroke have been studied far less.

GABAergic activity following stroke has been gauged using TMS [10], positron emission tomography (PET) [11], and pharmacological modulation [12]. However, neither PET nor TMS provide direct measures of GABA levels. They instead detect changes in receptor levels or combined receptor/transmitter changes. Recent GABA-related studies have employed MEscher-Garwood Point Resolved Spectroscopy (MEGA-PRESS) as a non-invasive MRS method sensitive to GABA levels in tissue [13]. For this cohort study of patients in acute stages of stroke, we similarly invoked MEGA-PRESS MRS to determine changes in GABA+ activity before and after a single month-long program of rehabilitation.

Methods

All protocols followed those outlined in the Declaration of Helsinki and were approved by Institutional Review Board of The First Affiliated Hospital of Soochow University Hospital (IRB No. 2019-069). Each patient granted informed consent for participation. This study was entered into the Chinese Clinical Trial Registry (ChiCTR1900028517). Reporting guidelines adhered to those proposed by the Consolidated Standards of Reporting Trials (CONSORT) group.

Sample size calculation

Calculation of sample size was based on a previous study [8] in which mean ± standard deviation (SD) values of pretraining GABA+: creatinine ratios were 0.33±0.06 in the test group and 0.42±0.08 in the healthy control group. Power analysis indicated a needed sample size of n=15 per group to achieve a statistical power of at least 90% (α=0.05). Given a 10-15% estimated loss rate, required sample sizes were no less than 17 test patients and 17 healthy controls.

Subjects

We recruited a total of 20 patients with strokes and impaired motor function from the Neurology Department of principle investigator's hospital. Inclusion criteria were as follows: (1) first incidence of ischemic stroke; (2) occurrence within 2 weeks of symptom onset; (3) unilateral upper-limb motor deficits; (4) subcortical unilateral infarct on brain magnetic resonance imaging (MRI); (5) 18-80 years of age; (6) right-handed dominance, according to Edinburgh Handedness Inventory [14]; (7) normal auditory function; and (8) completion of 30-min daily training tasks. Exclusion criteria were the following: (1) hemorrhagic stroke; (2) other underlying neurological diseases; (3) severe aphasia or cognitive impairment [15]; (4) prescribed or taken antidepressants or benzodiazepines; (5) associated cancer, lung or heart disease; and (6) contraindications to MRI, including pacemaker implantation or epilepsy. Twenty patients (men, 16; mean age, 51.60±15.243 years) were recruited, all right-handed (see **Table 1**). A group of age- and sex-matched healthy control subjects (men, 16, mean age, 52.65±7.849 years), all right-handed, were likewise scanned for purposes of comparison.

Clinical interventions

Enrollees participated in standardized upper-limb rehabilitation, regardless of group assignment. Daily programs included 30 min of task-oriented training, individualized motor tasks, and activities of daily living instruction. The daily sessions were conducted for 5 consecutive days per week and sustained for 4 weeks. Session content and intensity was determined by therapists in charge, who were blinded to other interventions and uninvolved in any assessments or data analysis.

Measurement data

Clinical assessments: Observations were made pre- and post-intervention in this longitudinal study. Regional GABA activity and upper-extremity motor function were appraised in test subjects to compare with age-matched controls. All patients with strokes underwent neurological and physical functional assessments. Stroke-related neurological disability was determined using the National Institutes of Health Stroke Scale (NIHSS); handicap status

Table 1. Patient characteristics

Patient	Age (y)	Gender	Educational (y)	Hypertension	Diabetes	Atrial fibrillation	Days Since Stroke	Stroke Location
1	37	1	16	Yes	Yes	No	2	Right basal ganglia
2	29	1	7	No	No	No	13	Right temporal, parietal, occipital, insular, basal ganglia
3	56	1	8	No	No	No	3	Right frontal lobe, basal ganglia
4	45	1	8	No	No	No	8	Left medulla
5	59	1	2	Yes	No	No	4	Left basal ganglia
6	48	1	9	No	No	No	2	Right basal ganglia
7	62	1	0	No	No	No	3	Left occipital lobe
8	56	1	8	Yes	No	No	4	Left basal ganglia
9	44	0	2	Yes	No	No	14	Left basal ganglia
10	65	1	0	Yes	No	No	9	Left basal ganglia, temporal and occipital lobe
11	66	0	8	Yes	No	No	2	Right basal ganglia
12	66	0	11	Yes	No	No	6	Left basal ganglia, frontal, temporal, parietal, occipital
13	62	1	3	Yes	No	No	6	Left basal ganglia
14	64	0	0	Yes	Yes	No	8	Left midbrain
15	50	1	2	No	Yes	No	7	Left basal ganglia
16	23	1	8	No	No	No	7	Left basal ganglia, frontal, temporal, parietal
17	40	1	16	Yes	No	No	2	Right basal ganglia
18	79	0	1	0	1	0	5	Left basal ganglia
19	62	1	3	1	0	0	4	Right basal ganglia
20	23	1	15	0	0	0	4	Right basal ganglia

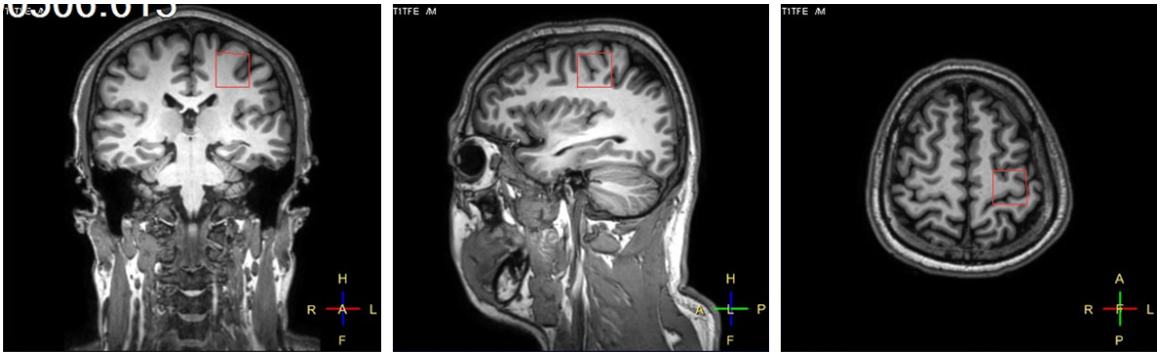

Figure 1. Example of magnetic resonance spectroscopy voxel placement in the left hemisphere.

was determined by modified Rankin scale (mRS); and limb motor function was based on Fugl-Meyer Assessment (FMA), examining both isolated and synergistic movements and limb strength (Grasping evaluation: 0, lowest score; 100, highest score) [16]. Upper-limb motor functions were further addressed by FMA for upper extremity (FMA-UE), again measuring isolated and synergistic movement patterns and grasping ability (0, lowest score; 66, highest score) [16]. The Barthel index (BI) was employed to profile global functionality (0, lowest score; 100, highest score) [17]. Two experienced evaluators maintained standard procedures for instrument calibration, data collection, and post-processing to ensure reproducibility and reliability of data [18, 19].

MRI assessments: Each patient submitted to Ingenia 3.0-Tesla scans (Philips Medical Systems, Amsterdam, Netherlands) prior to and following the 4-week training period. In healthy subjects, scans took place on a single occasion only. Structural T-weighted magnetization-prepared rapid gradient-echo (MPRAGE) and bilateral GABA-edited MRS studies were individually performed. Total scan times were close to 33 min. Prior to MRS scans, localization was achieved through T1-weighted 3D turbo field-echo (TFE) images, the major parameters configured as follows: time of repetition (TR), 6.9 ms; echo time (TE), 3.2 ms; slice thickness, 1 mm; matrix, 256*240; field of view (FOV), 256*256*185 mm^3; flip angle (FA), 8°; and pixel size (PZ), 1*1*1 mm^3. Knob controls for volume size of MRS voxels in bilateral hemispheres of patients and healthy subjects were set at 2.4*2.4*2.4 cm^3 [20] (**Figures 1** and **2**), sufficient to accommodate a voxel size of 13.824 cm^3. Voxels were strategically stationed to avoid lateral ventricle and skull [20, 21].

Proton magnetic resonance spectroscopy (^{1}H-MRS): GABA editing involved MEscher-Garwood Point RESolved Spectroscopy (MEGA-PRESS) sequences [22], configured as follows: TR, 2000 ms; TE, 68 ms; acquisition bandwidth, 2000 Hz; and scan duration, 13 min 20 sec. The J-evolution for GABA was refocused during odd-numbered acquisitions (ON), without commensurate even-numbered acquisition (OFF) adjustment. A Gaussian inversion pulse was applied to the ^{3}CH2 resonance of GABA at 1.9 ppm (ON) and symmetrically at a water peak of 7.5 ppm (OFF). For water suppression, chemical shift-selective (CHESS) pulses followed automated optimization settings. Prior to individual acquisitions, fast automatic shimming technique by mapping along projections (FASTMAP) was launched for VOI handling.

Edited spectra of GABA signaling were dictated by ON/OFF differences. All signals were measured at 3.02 ppm, which by MEGA-PRESS technique detects macromolecules (MM) and homocarnosine as well [23]. All signals were thus designated GABA+ (as opposed to GABA only).

^{1}H-MRS data processing: Quantifications were driven by the open-source Gannet 2.0 toolkit, a MATLAB-based (MathWorks, Natick, MA, USA) quantitative batch analysis tool enabling analysis of all GABA MEGA-PRESS spectra (**Figure 3**) [13]. There are two intrinsic Gannet software modules: Gannet Load and Gannet Fit. The Gannet Load module acquires specific variables from data headers, using a line broadening of 3 Hz and a frequency phase rooted in Spectral Registration to correct individual spectra [13] (**Figure 4**). The Gannet Fit applies a single Gaussian model to meet the edited GABA+ signal, which is then evaluated relative

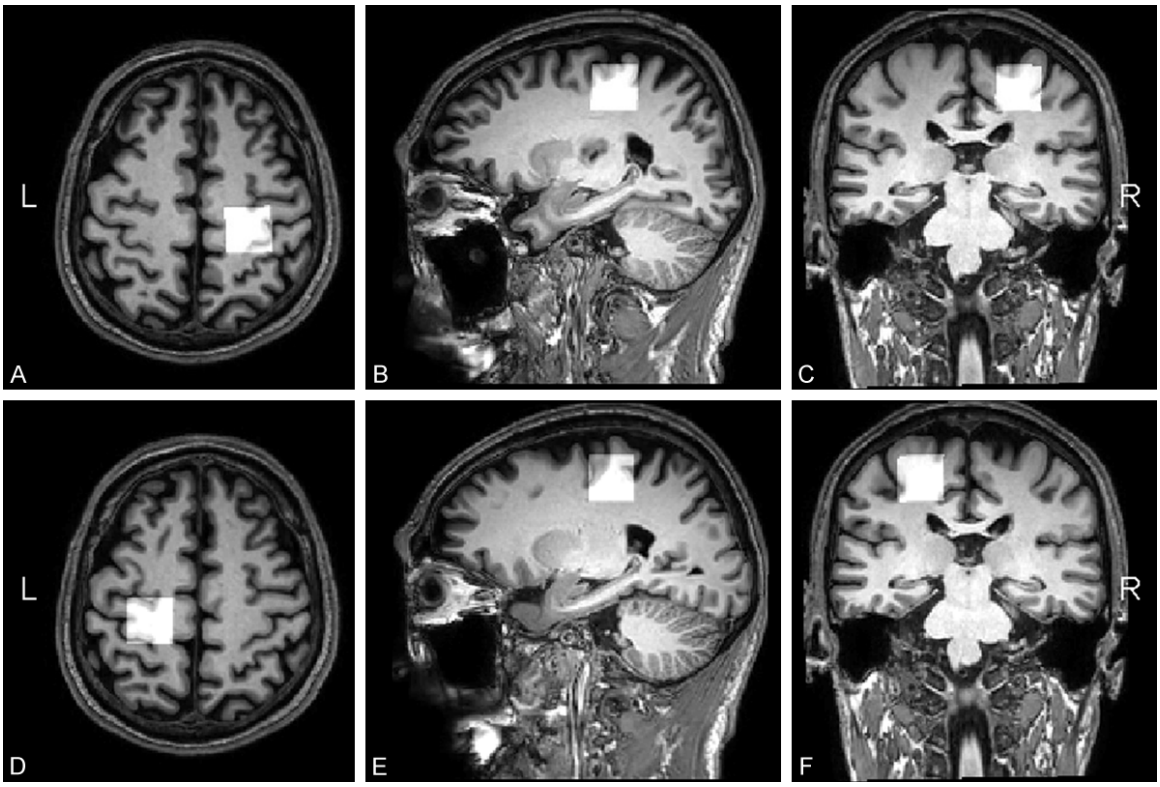

Figure 2. The position of volumes of interest (2.4 × 2.4 × 2.4 cm^3) in the right (above) and left M1 region (below) on sagittal (B, E) and coronal (C, F) T1-weighted images. The corresponding results of brain segmentation are shown for the right (A) and left auditory region (D). Abbreviations: M1, motor cortex.

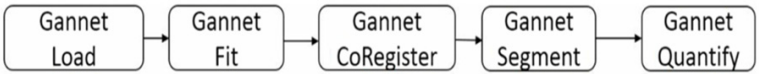

Figure 3. Flowchart of the MEGA-PRESS MRS data processing procedure.

to Cr levels (**Figure 5**). Of note, prior studies have indicated no changes in Cr levels during chronic phases of stroke [8]. Herein, we Permeating the blood brain barrier and abrogating the inflammation in stroke: implications for stroke therapy to GABA+/Cr ratios as GABA+ levels. Gannet Fit was implemented to divide SDs of fitting residuals by fitted peak amplitudes, generating overall Fit Errors that reflect signal-to-noise ratios. Only spectra with relative GABA+ Fit Errors ≤10% qualified for statistical analyses (**Figure 5**).

VOI segmentation: Metabolite determinations via ^{1}H-MRS may be impacted by voxel proportions of gray matter (GM), white matter (WM), and cerebrospinal fluid (CSF). We evaluated GABA+ concentrations in both test patients and healthy controls to accurately assess differing post-infarction tissue composition and gauge subsequent motor improvement. Each MRS voxel was segmented as GM, WM, or CSF, using 3D T1-weighted brain images and an automated brain segmentation program, Statistical Parametric Mapping (SPM8, *http://www.fil.ion.ucl. ac.uk/spm/*) [24] (**Figure 6**). Tissue GM fractions were thereby obtained as the ratio of GM to GM+WM volumes in VOIs. Concentrations of GABA and Cr in CSF were ultimately deemed negligible [25-29].

Statistics

All MRS data were expressed as mean ± SD values. In baseline comparisons of the two groups, GABA+/Cr ratios were analyzed by independent *t*-tests for data with normal distributions. Changes in GABA+/Cr ratios after training were assessed by paired *t*-test. Pearson's correlation coefficient was utilized to ascertain links between pre-intervention FMA scoring of motor status and pre-intervention GABA+/Cr

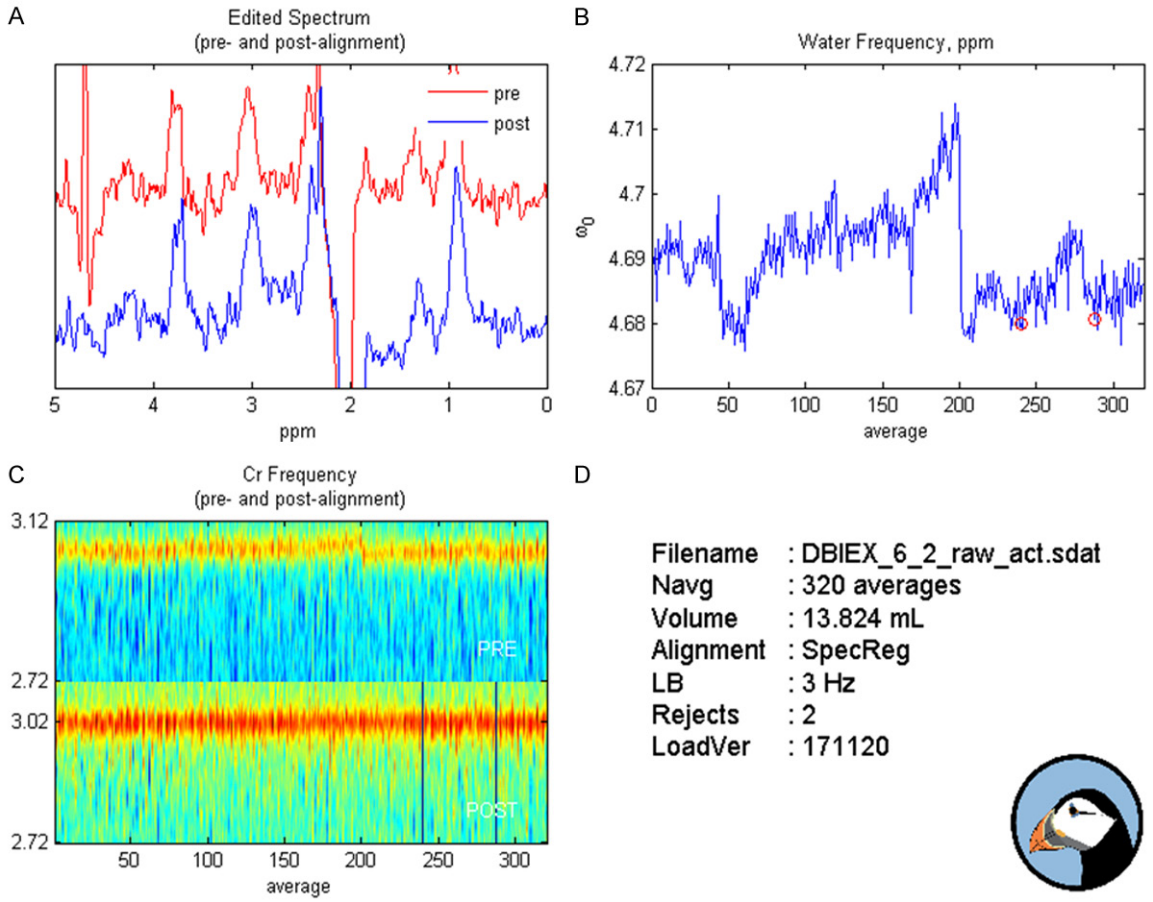

Figure 4. Gannet Load Output pdf files. A: Shows the processed GABA-edited difference spectrum, This plot shows the spectrum before frequency and phase correction above in red and the spectrum after frequency and phase correction below in blue. B: Shows the frequency of the maximum point in the spectrum (usually residual water signal) plotted against time. C: Presents the Cr signal over the duration of the experiment. The spectra at each timepoint are presented as a vertical stripe in the image, color-coded according to signal intensity, the Cr signal appears as a 'hot' stripe running through the image. D: Shows the filename and some descriptive variables.

fractions in affected hemispheres. Interventions aimed at functional recovery were examined in relation to baseline GABA+/Cr ratios of injured hemispheres, using simple regression. Qualitative FMA scores served as primary outcome measures. Standard software (SPSS v22.0; IBM Corp, Armonk, NY, USA) was engaged for statistical computations, setting significance at $P \leq 0.05$.

Results

Baseline GABA+ levels (prior to rehabilitation) were compared. The pretraining GABA+/Cr ratio in test patients (mean, 0.109±0.023) was significantly lower than that of healthy controls (mean, 0.135±0.017; t=-4.108; P=0.000) (**Table 3**; **Figure 7**). GM levels within voxels were comparable among patients (affected hemisphere: 24.393±5.570%; unaffected hemisphere: 24.504±6.088%) and among healthy subjects (left hemisphere: 24.791±4.400%; right hemisphere: 25.033±4.216%), and no significant group differences were observed (P>0.05) (**Table 3**). To address confounding changes in GABA+ determinations of dominant and non-dominant hemispheres, bi-hemispheric scanning was performed. Surprisingly, the mean GABA+/Cr ratio increased after training (0.119±0.014 vs 0.109±0.023; t=-2.143; P=0.045) (**Table 2**; **Figure 7**). There was a significant positive correlation between motor assessment by pre-intervention FMA and pre-intervention GABA+/Cr fractions in affected hemispheres (r=0.514; P=0.020) (**Figure 8**). After training, qualitative FMA scores also cor-

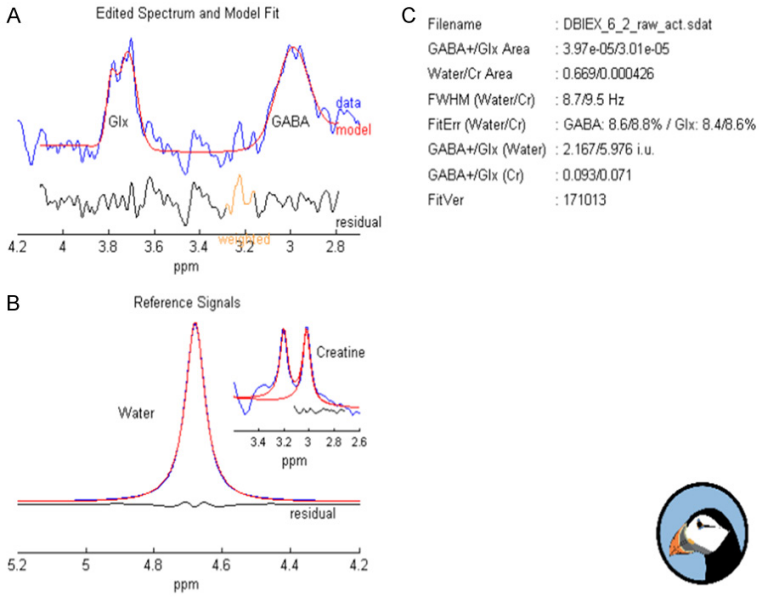

Figure 5. Gannet Fit Output pdf files. This pdf shows the curve-fitting of the GABA peak using Gannet, the red lines in the panels are the results of the Gannet Fit curve-fitting, the blue lines show the post phase and frequency aligned GABA data, and the black line is the residual difference between the experimental data and the curve-fit. A: Shows the modeling of the GABA signal. B: Shows the modeling of the signal against which GABA is quantified. C: Contains the results of the fitting.

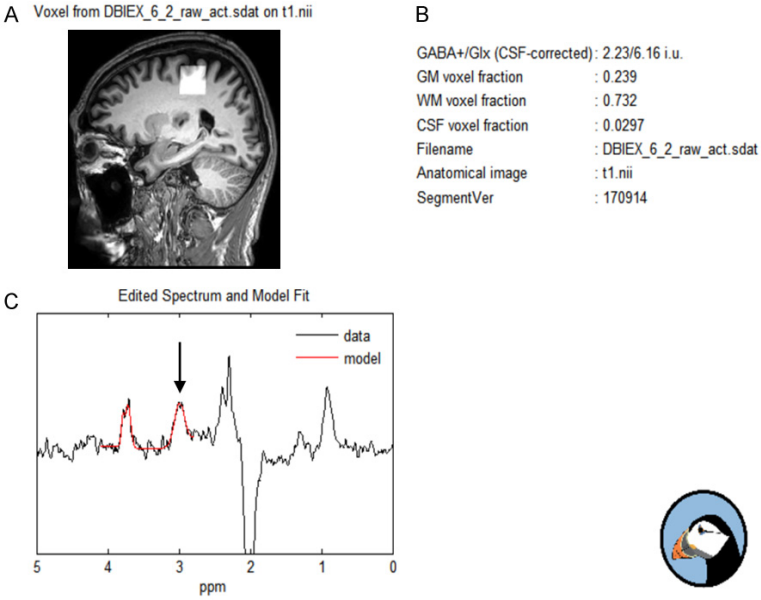

Figure 6. Example output from Gannet Segment: A: A single slice to show the voxel location. B: The CSF-corrected GABA concentration and the voxel tissue fractions are summarized. C: The filtered difference spectrum in a patient. Arrow indicating the GABA (γ-aminobutyric acid) peak at 3 ppm.

related significantly with fractional pretraining GABA+/Cr ratios (r=0.500; P=0.025) (**Table 4**; **Figure 9**), whereas GM changes within MRS voxels and functional improvement were unrelated (affected hemisphere: r=0.127 [P=0.592]; unaffected hemisphere: r=0.116 [P=0.626]) (**Table 4**). These findings suggest that fractional pretraining GABA+/Cr ratios of affected hemispheres, rather than GM volume changes, correspond with FMA scores after rehabilitative intervention (FMA1). The potential impact of fluctuating Cr on observed correlation between GABA+/Cr and FMA1 was thereby addressed.

Discussion

In this study, GABA+ levels measured in test patients (vs healthy controls) were found to decline significantly after strokes and were associated with subsequent rehabilitative motor improvements. This underscores the key role of GABA during recovery from stroke.

Decline in GABA+ levels following stroke

It is clear that GABAergic signaling mediates recovery from brain damage. There is demonstrable loss of GABA receptor expression due to rising ischemia-related extracellular GABA concentrations [30]. Declines in GABA(+)-related inhibition enhance use-dependent M1 plasticity [31], whereas GABA agonists (such as lorazepam) reduce use-dependent plasticity in the setting of brain damage [32]. *In vivo* studies have also shown that GABA agonist activation of GABAA receptors in medial motor cortex aggravates motor-learning error rates during motor sequence production [33]. So we can further infer that higher M1 levels of GABAA receptor functionality are then implicated in states of low-level motor function.

Table 2. Indicators prior to- and following treatment in patients group

Variables	Pre-training (n=20)	Post-training (n=20)	t value	*P* value
FMA	48.350±27.258	72.550±30.932	-7.084	0.000*
upper-limb FMA	30.600±21.493	45.950±24.267	-4.595	0.000*
NIHSS	6.500±3.887	3.350±3.150	6.842	0.000*
BI	53.500±22.130	87.000±20.157	-7.887	0.000*
mRS	3.650±0.813	2.000±1.124	7.906	0.000*
MMSE	22.950±8.463	25.2000±6.978	-1.966	0.064
affected hemisphere GABA: Cr	0.109±0.023	0.119±0.014	-2.143	0.045*
unaffected hemisphere GABA: Cr	0.111±0.030	0.117±0.123	-0.996	0.332
affected hemisphere GM/GM+WM	24.393±5.570	20.778±3.657	2.608	0.017*
unaffected hemisphere GM/GM+WM	24.504±6.088	22.863±5.554	0.920	0.369

Abbreviations: FMA, FuglMeyer Assessments; NIHSS, National Institutes of Health Stroke Scale; BI, Barthel index; mRS, modified Rankin scale; MMSE, Mini Mental State Examination; GABA, γ-aminobutyric acid; Cr, creatine; GM, proportions of gray matter; WM, white matter; affected hemisphere, M1 of the lesioned hemisphere; unaffected hemisphere, M1 of the nonlesioned hemisphere. *represent $P<0.05$.

Table 3. Comparison of GABA:Cr before treatment between patients and healthy subjects groups

Variables	Patients (n=20)	healthy subjects (n=20)	t value	*P* value
affected/left (GABA: Cr)	0.109±0.023	0.135±0.017	-4.108	0.000*
unaffected/right (GABA: Cr)	0.111±0.030	0.134±0.043	-2.017	0.051
affected/right hemisphere (GABA: Cr)	0.109±0.023	0.134±0.043	-2.291	0.028*
unaffected/left (GABA: Cr)	0.111±0.030	0.135±0.017	-3.265	0.002*
affected/right GM/GM+WM	24.393±5.570	25.033±4.216	-0.410	0.684
unaffected/left GM/GM+WM	24.504±6.088	24.791±4.400	-0.171	0.865
affected/left GM/GM+WM	24.393±5.570	24.791±4.400	-0.251	0.803
unaffected/right GM/GM+WM	24.504±6.088	25.033±4.216	-0.320	0.751

Abbreviations: GABA, γ-aminobutyric acid; Cr, creatine; GM, proportions of gray matter; WM, white matter; left, M1 of the left hemisphere; right, M1 of the right hemisphere; affected, M1 of the lesioned hemisphere; unaffected, M1 of the nonlesioned hemisphere. *represent $P<0.05$.

Past studies reporting drops in GABA activity after strokes have offered the following explanations: (a) selective loss of GABA neurons (in PET images) during ischemia, (b) disinhibition of functionality supporting motor recovery [34, 35] or (c) sustained motor function capability [12, 36]. Pharmacological research has linked GABAergic activity loss to continued motor function during chronic post-stroke stages, given that GABA agonists promote functional recovery loss after stroke [12]; and GABA loss within primary motor cortex has been demonstrated during chronic phases of stroke [21]. Such deficits in non-lesion-bearing prefrontal cortical regions of inpatients are encountered post-stroke during early and later stages (~3 months) [37]. Similar loss of resting GABA+ levels has been further documented via ppTMS [21]. In short-interval intracortical inhibition (SICI) assessments at varying delays, intercortical inhibition is reduced in ipsilesional (vs contralesional) M1 to similar extents in test subjects and controls [34]. However, TMS is more demanding in actual patients than in healthy controls, owing to the technical challenge of acquiring motor-evoked potentials (MEPs) in damaged corticospinal tracts. TMS assessments are therefore skewed towards well-recovered patients with measurable MEPs. Despite this problematic aspect of TMS, there is pooled published evidence of a global reduction in cortical inhibition, at least for changes exhibited during chronic stages of post-stroke recovery.

MRS affords a more direct means of measuring local GABA+ concentrations, having recently confirmed a lowering of GABA+ in chronic stages of stroke [37]. Nevertheless, the past focus on chronic-phase patients has ignored investigation of GABA+ within intact hemispheres over time. For the present study, we

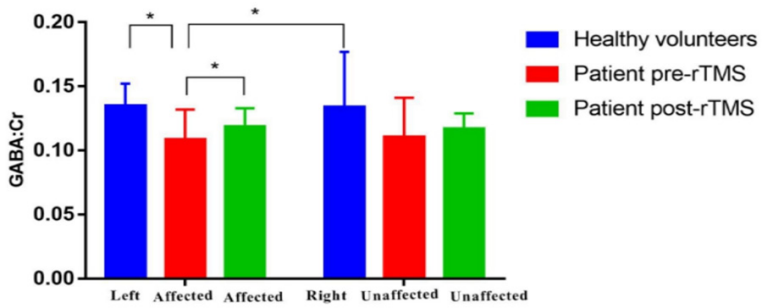

Figure 7. GABA: Cr levels (mean, SEM) was significantly lower in patients prior to treatment, compared to healthy subjects and following treatment. Abbreviations: GABA, γ-aminobutyric acid; Cr, creatine; SEM, standard error of the mean; Left, M1 of the left hemisphere; Right, M1 of the right hemisphere; Affected, M1 of the lesioned hemisphere; Unaffected, M1 of the nonlesioned hemisphere. *represent P<0.05.

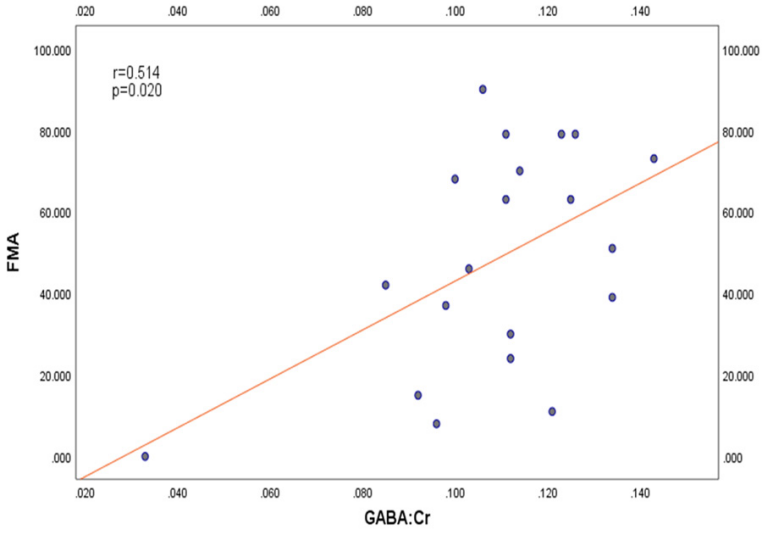

Figure 8. Correlation between FMA scores pre-intervention and pre-intervention GABA: Cr fractions. Note that there was a positive significant correlation between FMA scores pre-intervention and pre-intervention GABA: Cr fractions (*r*=0.514, *P*=0.020). Abbreviations: FMA, FuglMeyer Assessment; GABA, γ-aminobutyric acid; Cr, creatine.

used edited MRS values to explore bi-hemispheric shifts in GABA+/Cr, anticipating that the ratios of intact hemispheres and in healthy controls would exceed those of affected hemispheres. Compared with matched healthy subjects, we again found lower GABA+/Cr ratios in patients with acute strokes, so it is unlikely that low-level GABA+ in the aftermath of stroke is attributable to cortical atrophy alone. Although GABA+/Cr ratios of intact hemispheres surpassed ratios of affected hemispheres, the values did not differ significantly.

When measuring corticospinal and intracortical excitability by TMS, cortical excitability appears to be lower in affected hemispheres of patients with strokes [38]. Earlier efforts have emphasized the importance of GABAergic signaling in functional links between sensory and motor cortical regions that govern motor movements. Initial increases in GABAergic inhibition of motor cortex may reflect abnormal functional linkage of motor cortical points, limiting sensory input to receptive fields. Within networked cortical and subcortical regions of the motor system, there is interplay between excitatory and inhibitory circuitry, culminating in motor output. Balance within this network may be disturbed following strokes [39]. Herein, we observed declines in GABA+ levels, likely due to diminished functional inhibition that supports either recovery or sustained motor ability. Patients in this study displayed subcortical lesions, so selective loss of (cortical) GABAergic neurons may not account for the lower GABA+ levels detected.

On the other hand, it has been shown that short-interval intercortical inhibition as a surrogate of synaptic GABA activity declines in both hemispheres during functional recovery after stroke [12, 40]. Midazolam is a GABA agonist leading to clinical deficits following stroke that validates the clinical relevance of GABA-sensitive pathways during stroke recovery [12]. Our data have shown no difference in GABA+ levels of intact and affected hemispheres, and GABA+ measured in unaffected hemispheres of test patients was less than corresponding levels in healthy controls.

Relation between motor performance and GABA+ levels

The acute post-stroke period has been studied less in humans than in murine models. Despite

Table 4. Univariate Logistic Regression Analyses of FMA1

	Univariate		
	r^2	r	P
affected hemisphere GABA: Cr	0.250	0.500	0.025*
unaffected hemisphere GABA: Cr	0.000	0.004	0.950
affected hemisphere GM/GM+WM	0.016	0.127	0.592
unaffected hemisphere GM/GM+WM	0.013	0.116	0.626

Abbreviations: FMA1, FMA score after invention; GABA, γ-aminobutyric acid; Cr, creatine; GM, proportions of gray matter; WM, white matter; affected hemisphere, M1 of the lesioned hemisphere; unaffected hemisphere, M1 of the nonlesioned hemisphere. *represent $P<0.05$.

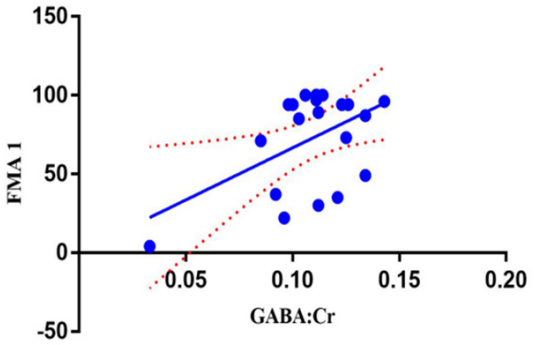

Figure 9. Elevated FMA scores post- intervention were associated with lower levels of pre-training GABA: Cr fractions. (r=0.500, P=0.025). Abbreviations: FMA, FuglMeyer Assessment; FMA1, FMA score after intervention; GABA, γ-aminobutyric acid; Cr, creatine.

ample profiling of GABAergic inhibition in the chronic phase of post-stroke motor function recovery, the relation between motor performance and GABA+ concentrations has yet to be similarly understood in acute phases of stroke. Our efforts have demonstrated a significant positive correlation between motor assessments of affected limbs and GABA+ concentrations of affected hemispheres at baseline. To our knowledge, this is the first *in vivo* proof of a relation between GABA+ concentration and motor function after acute cerebral infarction in humans, perhaps warranting use of GABA+ as a related neurochemical biomarker.

GABA+ changes in relation to motor recovery

GABA levels are known to fluctuate after strokes. In recently conducted PET studies, Kim et al. used Flumazenil (a GABA receptor antagonist) to assess cerebral GABA activity in 10 survivors of unilateral subcortical ischemic stroke, analyzed 1 or 3 months after occurrence [41]. At the 1-month mark, lower levels of GABAergic activity were observed relative to age-matched controls (n=15), although these levels rebounded after 3 months. It was reported that such infarcts produced loss of brain activation, with GABAergic activity in M1 expected to initially rise and then decline over time, based on findings of Cramer and colleagues [42]. Adding to the understanding of plasticity during stroke recovery, we have identified a significant correlation between motor recovery and pre-intervention GABA+ level. Our research suggests that GABA+ declines of primary motor cortex benefit patients in the acute phase of stroke recovery.

Technical considerations and study limitations

The sample size of each group (n=20) was rather small for a conventional clinical study. However, these subjects were required to lie quietly in the scanner for more than 30 min, with eyes closed. Some were uncooperative, prompting exclusion. Our data should thus be viewed as preliminary, necessitating confirmation in a trial of larger scale.

Because others have shown the stability of GABA+/Cr (up to 7 months) within occipital lobes of healthy subjects [43], our healthy controls were scanned on one occasion only, not expecting GABA+ to change. Also, random alterations in GABA+ would not readily explain our study observations. GABA+ levels are known to decline with age, but the subjects we recruited as healthy controls were matched by sex and age [44]. A MRS approach involving smaller VOIs may prove more efficient for region-specific brain analyses of GABA+ levels. As in most MRS studies of GABA, the voxel volume selected was relatively large (24 mm*24 mm*24 mm) due to the low intensity of GABA+ signals. The type of MRI system engaged no doubt has some impact in this regard.

Our study protocol did not incorporate acquisition of non-suppressed water scans, precluding the calculation of GABA+ concentrations relative to brain water signals. Results presented here were thus metabolite-restricted (Cr) ratios. However, referencing of Cr (rather than water) should allow more robust assessment of poten-

tial group differences in brain water content and CSF due to atrophy [45].

Finally, GABA+/Cr ratios tend to change during prolonged measurement periods (>40 min). In future studies, a larger sample size and further assessments of GABA dynamics are essential [24] to cement the relation between GABA signaling and recovery, providing a theoretical and practical basis for treatment efficacy.

Conclusion

In conclusion, we found that GABA+ levels of primary motor cortex decline in patients with acute strokes (compared with healthy subjects), showing a significant positive correlation with motor recovery. Overall, these outcomes underscore the importance of GABA in M1 disinhibition and early learned movement after stroke, serving as a key biomarker for motor recovery.

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Disclosure of conflict of interest

None.

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