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RESEARCH ARTICLE

Longitudinal assessment of ^{1}H-MRS (GABA and Glx) and TMS measures of cortical inhibition and facilitation in the sensorimotor cortex

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Abstract

The purpose of the present study was to investigate the long-term stability of water-referenced GABA and Glx neurometabolite concentrations in the sensorimotor cortex using MRS and to assess the long-term stability of GABA- and glutamate-related intracortical excitability using transcranial magnetic stimulation (TMS). Healthy individuals underwent two sessions of MRS and TMS at a 3-month interval. A MEGA-PRESS sequence was used at 3 T to acquire MRS signals in the sensorimotor cortex. Metabolites were quantified by basis spectra fitting and metabolite concentrations were derived using unsuppressed water reference scans accounting for relaxation and partial volume effects. TMS was performed using published standards. After performing stability and reliability analyses for MRS and TMS, reliable change indexes were computed for all measures with a statistically significant test–retest correlation. No significant effect of time was found for GABA, Glx and TMS measures. There was an excellent ICC and a strong correlation across time for GABA and Glx. Analysis of TMS measure stability revealed an excellent ICC for rMT CSP and %MSO and a fair ICC for 2 ms SICI. There was no significant correlation between MRS and TMS measures at any time point. This study shows that MRS-GABA and MRS-Glx of the sensorimotor cortex have good stability over a 3-month period, with variability across time comparable to that reported in other brain areas. While resting motor threshold, %MSO and CSP were found to be stable and reliable, other TMS measures had greater variability and lesser reliability.

Keywords Magnetic resonance · MEGA-PRESS · Motor cortex · Transcranial magnetic stimulation · GABA · Glutamate

Introduction

Proton magnetic resonance spectroscopy and transcranial magnetic stimulation are non-invasive techniques used to assess GABAergic and glutamatergic activity in the brain. Despite their parallel and complementary applications, studies have found that measurements obtained through both techniques do not correlate, and it was thus hypothesized that MRS and TMS have different neurochemical substrates and mechanisms of action (Stagg et al. 2011a; Tremblay et al. 2013; Dyke et al. 2017). Indeed, it is believed that MRS can directly measure extrasynaptic GABA and glutamate concentrations, and that TMS indirectly reflects GABA and glutamate receptor function (Stagg et al. 2011a).

MRS allows for direct in vivo quantification of various neurometabolites by taking advantage of their molecular properties. Depending on their chemical environment, protons interact differently with the magnetic field, giving rise to signals at characteristic chemical shifts at which corresponding peaks or set of peaks can be seen on the spectra. The location and area under the peaks provide information regarding the nature and concentration of the molecules of interest. By using J-difference spectral editing sequences such as MEGA-PRESS, which take advantage of J-couplings—interactions through the electron network of non-equivalent protons with shared chemical bonds within a molecule—it is possible to obtain a 3 ppm GABA signal (Mescher et al. 1998; Mullins et al. 2014; Rae 2014). Also

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using MEGA-PRESS, a composite glutamate/glutamine (Glx) signal at 3.75 ppm, comprising glutamate and glutamine resonances that cannot be resolved at moderate field strengths (≈3.0 T), can also be obtained (Mullins et al. 2014).

TMS has been used to modulate and probe neurophysiological mechanisms underlying cortical function and plasticity (Hallett 2007). When applied to the motor cortex, the TMS-triggered depolarization activates interneurons, leading to trans-synaptic activation of pyramidal cells, which in turn induces descending volleys in the corticospinal tract, where pyramidal axons project on spinal motoneurons (Klomjai et al. 2015). The subsequent motoneuron activation induces muscle responses called motor evoked potentials (MEP). Using different techniques, various TMS measures can be obtained and are believed to rely on different receptor-dependent physiological mechanisms reflecting inhibitory and excitatory processes in the brain.

Despite their distinct methodological properties, MRS and TMS can be used in a clinical setting to assess disease progression and treatment response (Cantello et al. 1991; Ziemann et al. 1997; Mills 2003). Indeed, abnormal GABA and glutamate signaling has been found to be implicated in various neurological and psychiatric conditions, such as amyotrophic lateral sclerosis, Parkinson's disease, and schizophrenia (Han and Ma 2010; Emir et al. 2012; Foerster et al. 2012; Rowland et al. 2012). Monitoring metabolite concentration using MRS can thus provide insight into the presence and evolution of disease and permit objective evaluation of treatment response. Furthermore, various pathological processes such as epilepsy, amyotrophic lateral sclerosis, Parkinson's disease, multiple sclerosis and cerebral lesions were found to alter TMS responses across a wide variety of measures (Mills and Nithi 1997; Rossini et al. 2015).

However, to increase the validity and use of MRS and TMS as diagnostic and disease-monitoring tools, the long-term stability and reliability of both techniques must be further examined. With respect to MRS, few studies have investigated the long-term stability of GABA or glutamate concentrations in healthy individuals (Near et al. 2014). As for TMS, while motor thresholds (MT) were found to be stable across a number of studies (Mills and Nithi 1997; Carroll et al. 2001; Ngomo et al. 2012; Hermsen et al. 2016), inconsistent results have been reported with regard to paired-pulse paradigms. Indeed, while some studies have found paired-pulse techniques (ppTMS) to be stable and reliable (Ngomo et al. 2012; Hermsen et al. 2016), others have reported high variability across sessions (Boroojerdi et al. 2000; Maeda et al. 2002; Wassermann 2002; Orth et al. 2003). Importantly, the long-term stability of TMS and MRS measures of inhibitory and excitatory activity has not yet been reported within the same individual. This is of significant interest to determine whether intraindividual variance of GABA/glutamate extrasynaptic concentration and receptor function share a common factor.

The goal of the present study was threefold. First, it aimed at assessing if single voxel MRS-GABA and Glx concentrations within the sensorimotor cortex are stable across time. Water was chosen as a reference instead of total creatine or *n*-acetylaspartate as it is readily quantifiable (Gasparovic et al. 2006) and may possess greater usefulness for expressing neurometabolite levels due to varying NAA and Cr concentrations in clinical conditions (Martin 2007). Second, it aimed at determining the long-term stability of various TMS measures (rMT, %MSO, SICI, ICF, LICI, CSP) that have been used in clinical settings. Third, it aimed at further exploring the ambiguous link between MRS and TMS measures of GABA and glutamate and determining whether intraindividual variation in one technique can predict variation in another.

Methods

The study consisted of two MRI sessions lasting approximately 60 min immediately followed by TMS sessions lasting approximately 30 min at a 3-month interval (t_1 and t_2).

Participants

Fourteen healthy right-handed participants (8 male, 6 female) aged 18–40 years were recruited using advertisements posted on campus and social media. Exclusion criteria were the following: neurological or psychiatric conditions, psychoactive medication (past or present intake), history of traumatic brain injury, history of fainting or seizures, substance abuse, and any contraindications to MR scanning or transcranial magnetic stimulation. All participants provided written informed consent prior to testing, and the experiments were performed with the approval of the local ethics committee (*Comité mixte d'éthique de la recherche du RNQ*). Participants were instructed to refrain from alcohol consumption 48 h before each session and from consumption of psychoactive drugs for the duration of the study. Two participants abandoned the study after one session, and one was excluded after the second session due to an anatomical anomaly. Their data were excluded from the final sample.

Magnetic resonance imaging

Magnetic resonance imaging sessions were performed at the Unité de Neurimagerie Fonctionnelle, Centre de Recherche de l'Institut Universitaire de Gériatrie de Montréal. MR acquisitions were performed using a 3 T whole-body system scanner (MAGNETOM Trio, Siemens, Erlangen, Germany) using a 32-channel receive-only head coil.

Anatomical images were acquired using a T_1-weighted MPRAGE sequence according to the following parameters: T_R (repetition time) = 2300 ms; T_E (echo time) = 2.98 ms; FA (flip angle) = 90°; FOV (field of view) = 256 mm, matrix = 256 × 256 × 176; T_I (inversion time) = 900 ms; number of slices = 176; slice thickness = 1 mm; orientation: sagittal; voxel size 1.0 × 1.0 × 1.0 mm^3; acquisition time 9:50 min.

Magnetic resonance spectroscopy

Magnetic resonance spectroscopy was performed according to previously published procedures. (Lefebvre et al. 2018). Data were acquired by first manually placing a voxel of interest (30 × 30 × 30 mm^3, Fig. 1) over the left sensorimotor area according to published anatomical landmarks (Fig. 1; Yousry et al. 1997). Voxel placement at t_2 was performed using images showing voxel placement at t_1 with axial, coronal and sagittal views to ensure adequate stability throughout sessions. Shimming was done using FAST(EST)MAP (Gruetter and Tkác 2000) to ensure that the linewidth of water was under 10 Hz. A MEGA-PRESS sequence (Mescher et al. 1996, 1998) was used to acquire neurometabolite signals according to the following parameters: T_R = 3000 ms; T_E = 68 ms; Excite FA = 90°; Refocus FA = 180°. Double-banded pulses were used to simultaneously suppress water signal and edit the 3 ppm GABA γ-CH_2 resonance. The water-suppressing band was applied at 4.7 ppm, while the editing band was applied at 1.9 ppm (EDIT ON) or at 7.5 ppm (EDIT OFF). Additional water suppression using variable power with optimized relaxation delays (VAPOR) and outer volume suppression (OVS) techniques (Tkáč et al. 1999), optimized for the human 3 T system, were incorporated prior to running the MEGA-PRESS sequence. The acquisition frequency was centered on GABA at 3 ppm (delta frequency = − 1.7 ppm). To minimize frequency drift and maintain editing efficiency, MEGA-PRESS data were acquired in blocks of 32 'EDIT OFF' and 32 'EDIT ON' interleaved scans with frequency adjustments performed before each block. Four blocks were acquired for a total acquisition time of 12 min. Individual FIDs were stored for off-line processing. The water reference required for absolute metabolite quantification was obtained from a separate acquisition using the same MEGA-PRESS sequence and voxel prescription, but without MEGA and VAPOR water suppression (both set to "only RF off") and centered on water at 4.7 ppm (delta frequency = 0). A single block of four averages was acquired (acquisition time: 42 s).

Frequency and phase of individual averages were corrected off-line and then averaged, independently for 'EDIT OFF' and 'EDIT ON', to produce the 'EDIT OFF' and 'EDIT ON' subspectra. Small frequency errors between the 'EDIT OFF' and 'EDIT ON' subspectra were manually corrected by minimizing subtraction error in the difference spectra around the 3.9-ppm creatine and the 3.2-ppm choline resonance. The final difference spectra ('EDIT DIFF') were obtained by subtracting the 'EDIT OFF' from the 'EDIT ON' subspectra.

MRS data analysis

Both 'EDIT OFF' and 'EDIT DIFF' subspectra were analyzed using LCModel 6.2-1A, which calculated the best fit for these spectra as a linear combination of model spectra (Provencher 1993, 2001). The basis set for the 'EDIT OFF' spectra comprised an experimentally measured metabolite-nulled macromolecular spectrum acquired from the occipital region of an independent cohort of 11 healthy adults (no medical, neurological, or psychiatric conditions and not receiving medication) as well as simulated metabolite spectra. A MATLAB-operated home-written software based on density matrix formalism (Henry et al. 2001) was used to simulate the basis set for 'EDIT OFF' metabolite spectra according to known chemical shifts and J-couplings (Govindaraju et al. 2000). The basis set comprised simulated spectra of the following metabolites: alanine, ascorbate, aspartate, creatine (CH_2 moiety), creatine (CH_3 moiety), GABA, glucose, glutamate (Glu), glutamine (Gln), glycerophosphorylcholine, glycine, glutathione, lactate, myo-ionositol, *N*-acetylaspartate, *N*-acetylaspartylglutamate,

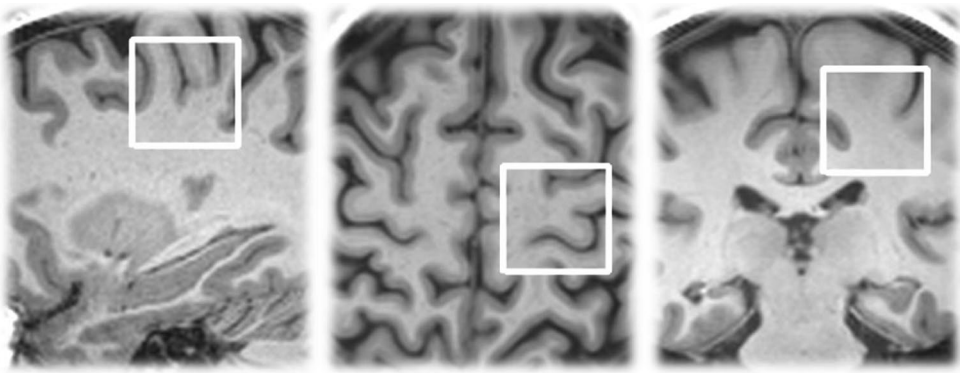

Fig. 1 Position of the voxel of interest (30 × 30 × 30 mm^3) over the left sensorimotor cortex

phosphocreatine (CH_2 moiety), phosphocreatine (CH_3 moiety), phosphorylcholine, phosphorylethanolamine, scyllo-inositol, and taurine. LCModel fitting was performed across the 0.2–4.0 ppm range for the 'EDIT OFF' spectra. The basis set for 'EDIT DIFF' difference spectra included an experimentally measured metabolite-nulled macromolecular (MM) spectrum from the occipital region (averaged across 11 subjects) as well as experimentally measured spectra from 100 mM NAA, GABA, Glu, and Gln phantoms at a 7.2 pH and at 37 °C. Fitting was performed over the 0.5–4.0 ppm spectral range for the 'EDIT DIFF' spectra. The occipital region was chosen for both the 'EDIT OFF' and 'EDIT DIFF' basis sets, because of its high signal-to-noise ratio. An example fitted difference spectra can be seen in Fig. 2. LCModel spline baseline modeling was deactivated for both 'EDIT OFF' and 'EDIT DIFF' spectra analysis with the NOBASE = T input parameter. Default LCModel simulations of lipid and MM resonances were also deactivated. No baseline correction, zero filling, or apodization functions were applied to the in vivo data prior to LCModel analysis. The independently acquired water signal was used as an internal standard reference for metabolite quantification. Spectra with GABA Cramér–Rao lower bounds (CRLB) > 30% were excluded from further analysis.

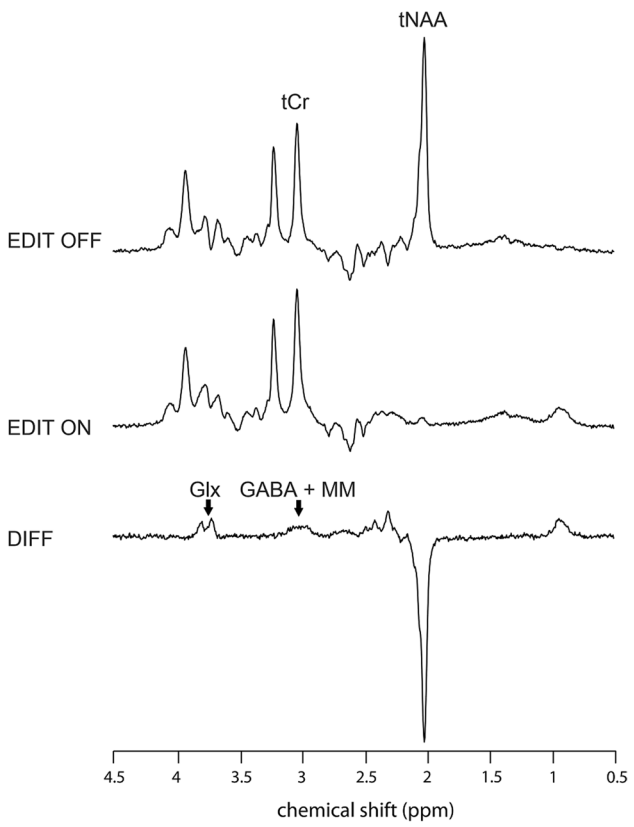

Fig. 2 Fitted spectra for EDIT OFF, EDIT ON, and DIFF spectra. The characteristic peaks of Glx and GABA + MM are shown

For the correction of relaxation and partial volume effects on water-referenced metabolite concentrations, the proportion of gray matter (GM), white matter (WM), and cerebrospinal fluid (CSF) within the MRS voxel was obtained following tissue segmentation performed on anatomical MPRAGE images from each participant using the automated *FreeSurfer* pipeline (V 5.3.0). The T_1 and T_2 water relaxation times used in the calculation of attenuation factors were taken from published reports [T_1 (GM) = 1.29 s, T_1 (WM) = 0.87 s, T_1 (CSF) = 4 s, T_2 (GM) = 110 ms, T_2 (WM) = 80 ms, and T_2 (CSF) = 400 ms] (Wansapura et al. 1999; Rooney et al. 2007). Water attenuation was computed using the fractional volume of each compartment (Gasparovic et al. 2006).

Water-referenced GABA, Glu, Gln, NAA, and MM values were obtained based on the segmentation-corrected 'EDIT DIFF' output (referred to as GABA/H_2O, Glu/H_2O, Gln/H_2O, NAA/H_2O, and MM/H_2O). As glutamate cannot be resolved from glutamine at 3 T, Glx/H_2O values were computed (Glu/H_2O + Gln/H_2O) and interpreted as an indicator of glutamate concentrations. GABA/Glx ratios, which reflect the balance of inhibitory and excitatory neurotransmitter concentrations, were also calculated as follows: (GABA/H_2O)/(Glx/H_2O) = GABA/Glx.

For better comparison with other studies, we also expressed metabolite concentrations as ratios to NAA and total creatine (tCr). For ratios to NAA (GABA/NAA and Glx/NAA), 'EDIT DIFF' water-referenced concentrations were divided by the 'EDIT DIFF' water-referenced NAA concentrations, following: GABA/NAA = (GABA/H_2O)/(NAA/H_2O) and Glx/NAA = (Glx/H_2O)/(NAA/H_2O). For ratios to tCr, water-referenced concentrations were divided by 'EDIT OFF' water-referenced tCr concentrations. Total creatine followed: tCr/H_2O = Cr/H_2O + PCr/H_2O, where Cr/H_2O = ($CrCH_3$/H_2O + $CrCH_2$/H_2O)/2 and PCr/H_2O = ($PCrCH_3$/H_2O + $PCrCH_2$/H_2O)/2, since the CH_2 and CH_3 moieties of creatine (Cr) and phosphocreatine (PCr), producing the 3.9-ppm ($CrCH_2$ and $PCrCH_2$) and 3.0-ppm ($CrCH_3$ and $PCrCH_3$) peaks, were fitted and quantified separately with four basis spectra ($CrCH_3$, $CrCH_2$, $PCrCH_3$, and $PCrCH_2$). GABA and Glx ratios to tCr therefore followed: GABA/tCr = (GABA/H_2O)/(tCr/H_2O) and Glx/tCr = (Glx/H_2O)/(tCr/H_2O), where Glx/H_2O and GABA/H_2O were obtained from 'EDIT DIFF' subspectra, for internal consistency and better comparison to other studies.

Transcranial magnetic stimulation

During TMS experiments, participants were seated comfortably on a chair, and instructed to remain relaxed, alert, still, and to keep their hands and feet uncrossed and palms facing slightly upward. Electromyographic (EMG) activity was recorded using two self-adhesive electrodes placed on

the right first dorsal interosseous (FDI) muscle and the side of the index finger to measure muscle contraction. A ground electrode was positioned over the right forearm muscle. The EMG signal was filtered with a bandwidth of 20–1000 Hz and digitized at a sampling rate of 4 kHz with a Powerlab 4/30 system (ADInstruments, Colorado Springs, CO, USA). Motor evoked potentials (MEPs) were recorded with Scope v4.0 software (ADInstruments, Colorado Springs, CO, USA) and stored off-line for analysis.

TMS was delivered over the left primary motor cortex through an 8-cm figure-of-eight coil connected to a MagPro stimulator (MagVenture, Farum, Denmark). The coil was positioned flat on the head of participants with a 45° angle from the midline, with the handle pointing backward to deliver biphasic currents in an anterior–posterior direction in the coil. Throughout the experiment, TMS pulses were delivered at a frequency of 0.1–0.2 Hz to avoid long-lasting modulation of M1 excitability (Chen et al. 1997). Resting motor threshold, paired-pulse and cortical silent period protocols were performed during both experimental sessions using published protocols (Rossini et al. 2015). The optimal site of stimulation was defined as the coil position from which TMS produced MEPs of maximum amplitude in the target muscle of the contralateral hand and marked on the participant's scalp using a water-soluble wax crayon to ensure stable coil positioning throughout the experiment.

Resting motor threshold

The resting motor threshold (rMT) was defined as the minimum stimulus intensity required to elicit MEPs of at least 50 μV in five of ten trials in a resting muscle.

Paired-pulse measures

Paired-pulse stimulation was performed with a test stimulus (TS) intensity that elicited MEPs ranging from ≈0.6 to 1.2 mV amplitude and a conditioning stimulus (CS) intensity set at 70% rMT. For short-interval intracortical inhibition (SICI) and intracortical facilitation (ICF), ten CS–TS pairs and ten TS-only pulses were delivered at different interstimulus intervals (ISI) in a randomized order for each participant. SICI was assessed at ISI_{2ms} and ISI_{3ms} (referred to as $SICI_{2ms}$ and $SICI_{3ms}$ throughout the text), and ICF was assessed at ISI_{9ms} and ISI_{12ms} (referred to as ICF_{9ms} and ICF_{12ms} throughout the text). The percentage of maximum stimulator output (%MSO) required to elicit MEPs of 1 mV average amplitude for the test stimuli was used as a marker of corticospinal excitability. For long interval intracortical inhibition (LICI), two successive pulses at TS intensity were delivered at an ISI_{100ms} until ten EMG recordings, where the first MEP had a peak-to-peak amplitude between 0.5 and 1.5 mV were obtained and recorded.

Cortical silent period (CSP)

To induce a CSP, a single TMS pulse with an intensity equivalent to 120% rMT was delivered while participants maintained a voluntary isometric muscle contraction of the right FDI at ≈20% maximum strength. To determine contraction strength, EMG signals were monitored as participants were first asked to briefly maintain maximum isometric muscle contraction while grasping a pencil with the thumb and index finger, and then relax until the peak-to-peak EMG signal amplitude was approximately 20% of the maximum. Participants were instructed to maintain this level of muscle contraction and were continuously instructed to increase or decrease muscle contraction, as needed, to ensure adequate stability of the tonic EMG signal.

Data analysis was performed in the same way at both time points (t_1 and t_2). MEPs (test stimulus, ISI_{2ms}, ISI_{3ms}, ISI_{9ms}, ISI_{12ms}, ISI_{100ms}) were visually inspected and trials with EMG activity reflecting muscle contraction in the 500 ms prior to stimulation were excluded from analysis. Outlier values (±3 SD) were also excluded. After averaging peak-to-peak MEP amplitudes for the TS-alone (TS-MEP) and paired-pulse measures, inhibition and facilitation indexes were computed as the ratio of the average MEP amplitude for each ISI ($SICI_{2ms}$, $SICI_{3ms}$, ICF_{9ms}, ICF_{12ms}) over the average TS-MEP amplitude. For LICI, the ratio of the average peak-to-peak amplitude of the second MEPs elicited over the average peak-to-peak MEP amplitude at test stimulus intensity was computed. CSP duration was measured manually from TMS pulse to resumption of sustained EMG activity, as shown in Fig. 3 (Groppa et al. 2012). Outliers (±3 SD) and CSPs without a clear delimitation were excluded, and the remaining CSP durations were averaged.

Statistical analysis

To verify uniformity of voxel placement across sessions, a repeated measures *t* test as well as a test–retest Pearson's correlation and intra-class correlation (two-way mixed

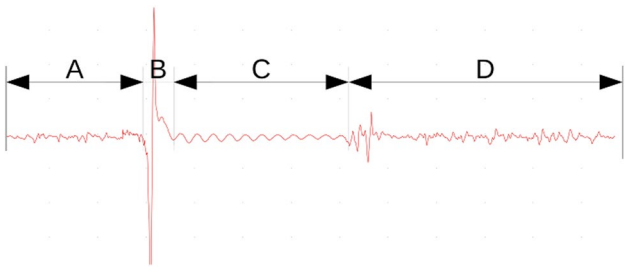

Fig. 3 EMG signal for the cortical silent period. **a** Period of tonic muscle contraction maintained at approximately 20% of maximum contraction. **b** MEP elicited from a TMS pulse at 120%rMT. **c** Period of EMG inactivity. **d** Resumption of tonic EMG activity

model) of the absolute agreement of single measures across time was performed for %GM, %WM, and %CSF. Coefficients of variation $\left(\text{COV} = \frac{\text{SD}}{\text{M}} \times 100\%\right)$ were computed separately across time (intra-subject) and across subjects (inter-subject) to describe within- and between-subject variability for GABA, Glx, GABA/Glx, and MM water-referenced metabolite levels, rMT, %MSO, TS-MEP, and CSP measures, as well as all paired-pulse ($SICI_{2ms}$, $SICI_{3ms}$, ICF_{9ms}, ICF_{12ms}, $LICI_{100ms}$) ratios. Despite not being the primary focus of this study, the same descriptive and reliability statistics were computed for water-referenced NAA and tCr, as well as NAA- and tCr-referenced GABA and Glx concentrations. Furthermore, a repeated measures *t* test was performed for these same variables to test for systematic effects. Repeated measures *t* tests were also performed to compare the test stimulus average MEP amplitudes and each of the average MEP amplitudes for $SICI_{2ms}$, $SICI_{3ms}$, ICF_{9ms}, ICF_{12ms}, and $LICI_{100ms}$ to assess paired-pulse effects on MEPs.

Test–retest reliability was assessed using Pearson's correlations between t_1 and t_2. Intra-class correlation coefficients (ICC) of the absolute agreement for metabolites of interest and TMS single measurements across time were computed using a two-way mixed model. ICC values were classified as poor (< 0.40), fair (0.40–0.59), good (0.60–0.74), or excellent (0.75–1.00) based on accepted guidelines (Cicchetti 1994; McGraw and Wong 1996). Reliable change indexes (RCI), which indicate the change in the quantitative variable that can be expected by random variation, were computed for MRS and TMS measures, with the test–retest correlation used as the coefficient of reliability. The following formula was used to determine RCI:

$$\text{RCI} = \sqrt{2 \times (\text{standard error of measurement})^2},$$

$$\text{Standard error of measurement} = \text{SD} \times \sqrt{1 - \text{coefficient of reliability}}.$$

Correlations were computed between water-referenced metabolite concentrations (GABA, Glx, GABA/Glx) and TMS measures (rMT, %MSO, SICI–ISI_{2ms}, SICI–ISI_{3ms}, ICF–ISI_{9ms}, ICF–ISI_{12ms}, $LICI_{100ms}$, and CSP) at t_1 and t_2 to assess the relationship between MRS and TMS measures. An analysis of the correlations between the COVs of MRS and TMS measures as well as between GABA and Glx levels and the ratio of the rMT needed to produce MEPs averaging 1 mV (%MSO/%rMT) was also performed.

Statistical analyses were performed using a standard statistical software package (SPSS 24, IBM, NY, USA). A *p* value ≤ 0.05 was considered statistically significant, and a Bonferroni correction was applied to correct for multiple comparisons.

Results

Following analysis, one subject with CRLB = 37% was excluded from the sample. The final sample comprised ten right-handed adults (5 males, 5 females), aged 19–35 (26 ± 4) years. Session 1 and 2 were separated by an average of 96 ± 7 days. Outlier data for one subject at ISI_{12ms} and another subject at ISI_{100ms} were excluded.

Magnetic resonance spectroscopy

The averages and standard deviations of CSF, GM, and WM percentages at t_1 and t_2 are shown in Table 1. Paired samples *t* tests revealed no significant effect of time for CSF ($t_{(9)} = 0.923$, $p = 0.380$), GM ($t_{(9)} = 0.021$, $p = 0.983$), and WM ($t_{(9)} = 0.112$, $p = 0.913$) ratios. Strong test–retest correlations and good to excellent ICCs were obtained for CSF ($r_{(10)} = 0.852$, $p = 0.002$; $r = 0.854$, 95% CI [0.539, 0.961], $F_{(9, 9)} = 12.530$, $p < 0.001$), GM ($r_{(10)} = 0.673$, $p = 0.033$; $r = 0.675$, 95% CI [0.090, 0.909], $F_{(9, 9)} = 4.738$, $p = 0.015$), and WM ($r_{(10)} = 0.741$, $p = 0.014$; $r = 0.749$, 95% CI [0.246, 0.932], $F_{(9, 9)} = 6.386$, $p = 0.005$) percentages. These results suggest that voxel positioning did not significantly differ between sessions.

Table 2 shows the mean (M) and the standard deviation (SD) across subjects of water-, NAA-, and creatine (tCr)-referenced metabolite values for each session. Average CRLBs were 18.6 ± 5.6 (range 12–29) for GABA, 2.90 ± 0.32 (range 2–3) for Glu, and 12.7 ± 2.8 (range 9–18) for Gln at t_1, and 17.4 ± 54 for GABA (range 11–26), 3.00 ± 0 for Glu (range 2–3), and 12.5 ± 1.3 (range 9–18) for Gln at t_2.

Paired samples *t* tests revealed no significant effect of time for GABA/H_2O ($t_{(9)} = 1.136$, $p = 0.285$), Glx/H_2O ($t_{(9)} = 1.163$, $p = 0.275$), and MM/H_2O ($t_{(9)} = 0.088$, $p = 0.931$) levels and GABA/Glx ($t_{(9)} = 1.838$, $p = 0.099$) ratios. Descriptive and reliability statistics for the main metabolites of interest (GABA/H_2O, Glx/H_2O), MM/H_2O, GABA/Glx ratios, and supplementary metabolites (NAA/H_2O, tCr/H_2O, GABA/NAA, Glx/NAA, GABA/tCr, Glx/tCr) are shown in Table 2. Furthermore, GABA/H_2O presented a strong test–retest correlation ($r_{(10)} = 0.815$, $p = 0.004$) and an excellent ICC ($r = 0.809$, 95% CI [0.432, 0.948], $F_{(9, 9)} = 9.715$, $p \leq 0.001$), Glx/H_2O presented a strong test–retest correlation ($r_{(10)} = 0.741$, $p = 0.014$) and a good ICC ($r_{(10)} = 0.641$, 95% CI [0.105, 0.895], $F_{(9, 9)} = 4.693$, $p = 0.015$), and MM/H_2O presented a non-significant test–retest correlation ($r_{(10)} = 0.353$, $p = 0.317$) and a poor ICC ($r_{(10)} = 0.374$, 95% CI [−0.368, 0.803], $F_{(9, 9)} = 2.075$, $p = 0.146$) (Fig. 4). GABA/Glx ratios presented a strong test–retest correlation ($r_{(10)} = 0.832$, $p = 0.003$) and an excellent ICC ($r = 0.780$, 95% CI [0.339, 0.940], $F_{(9, 9)} = 9.797$, $p \leq 0.001$). Intra- and inter-subject average COVs and RCIs

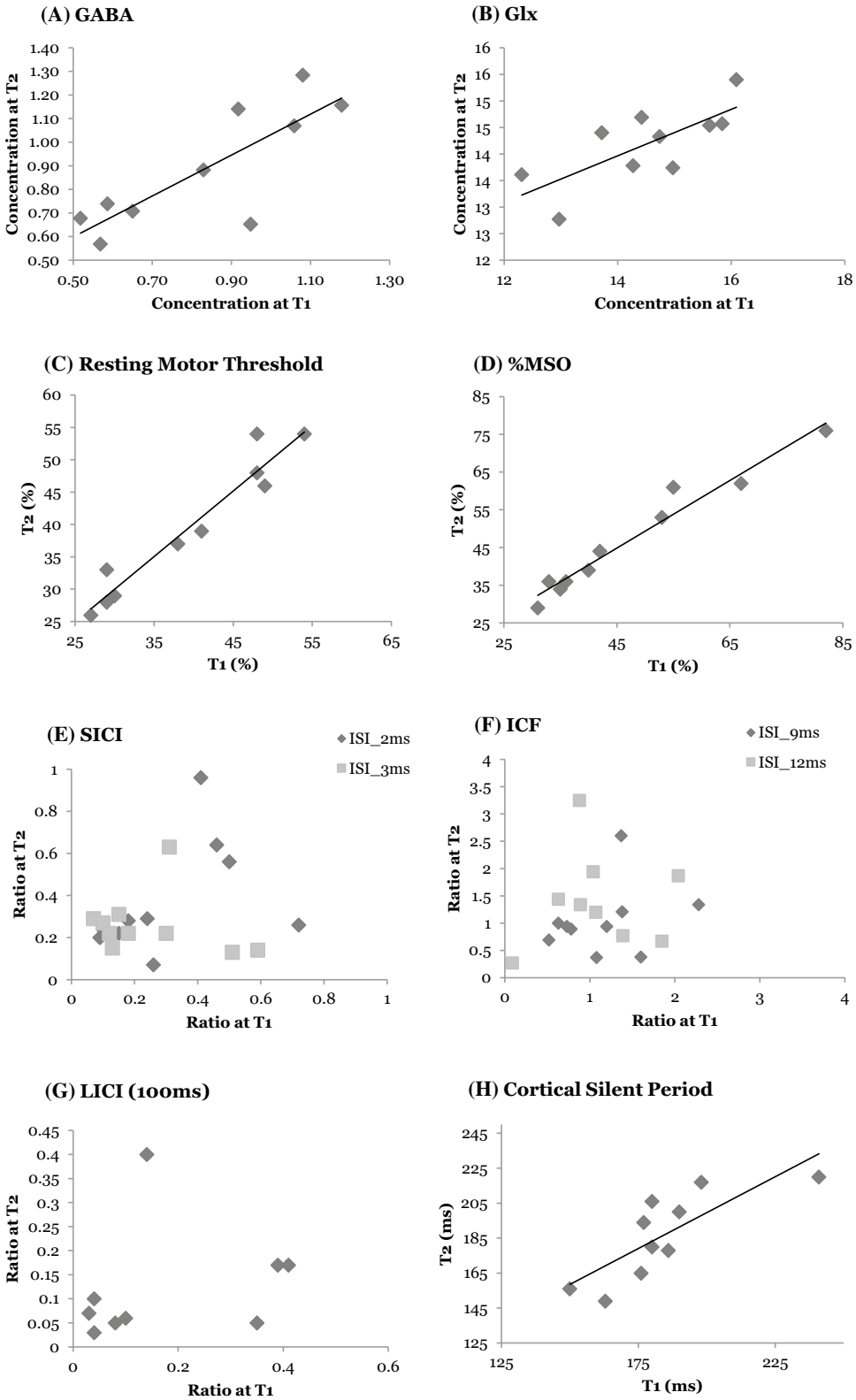

Fig. 4 Scatter plots illustrating the association between measures for MRS for **a** GABA, **b** Glx and TMS for **c** RMT, **d** %MSO, **e** SICI, **f** ICF, **g** LICI, and **h** CSP. Statistically significant correlations are identified by a trendline

are also shown in Table 2. In general, inter-subject COVs were larger than intra-subject COVs. It can also be seen that stability and reliability statistics for NAA- and tCr-referenced metabolites are generally equivalent to or poorer than H_2O-referenced metabolite values.

Table 1 Corticospinal fluid (CSF), gray matter (GM), and white matter (WM) ratios

	Time 1 (M ± SD)[a]	Time 2 (M ± SD)[a]	P[b]	r[c]	ICC[d]	RCI
CSF	0.033 ± 0.010	0.035 ± 0.010	0.38	0.85**	0.85**	0.006
GM	0.224 ± 0.040	0.224 ± 0.052	0.98	0.67*	0.68*	0.036
WM	0.743 ± 0.048	0.742 ± 0.058	0.91	0.74*	0.75**	0.037

*$p \leq 0.05$; **$p \leq 0.01$

[a]The sum of average (M) ratios may not be exactly equal to 1.00 due to rounding

[b]p value of the repeated measures t test

[c]Pearson's correlation coefficient between time 1 and time 2

[d]Intra-class correlation coefficient of the absolute agreement between single measures of time 1 and time 2 using a two-way mixed model

Table 2 Descriptive, stability, and reliability statistics for MRS and TMS variables

	Time 1 (M ± SD)	Time 2 (M ± SD)	Within-subject COV (%)	Between-subject COV(%)	r[a]	ICC[b]	RCI
Segmented MRS measures							
[GABA/H_2O]	0.834 ± 0.239	0.888 ± 0.254	10	29	0.815**	0.809**	0.147
[Glx/H_2O]	14.495 ± 1.232	14.183 ± 0.728	4	7	0.741*	0.641*	0.718
[NAA/H_2O]	22.268 ± 0.470	22.170 ± 0.758	1	3	0.722*	0.661*	0.459
[MM/H_2O]	4.462 ± 0.212	4.455 ± 0.185	3	4	0.353	0.374	0.220
[GABA/Glx]	0.057 ± 0.014	0.062 ± 0.017	10	25	0.832**	0.780**	0.009
[GABA/NAA]	0.037 ± 0.011	0.040 ± 0.012	11	30	0.798**	0.788**	0.007
[Glx/NAA]	0.652 ± 0.062	0.641 ± 0.044	3	8	0.703*	0.676*	0.041
[tCr/H_2O]	17.259 ± 1.647	17.695 ± 0.857	4	7	0.398	0.330	1.424
[GABA/tCr]	0.049 ± 0.016	0.050 ± 0.015	14	31	0.651*	0.669*	0.013
[Glx/tCr]	0.850 ± 0.137	0.803 ± 0.050	6	11	0.614	0.379	0.091
TMS measures							
rMT (%)	39 ± 10	39 ± 11	4	26	0.965**	0.968**	3
%MSO (%)	47 ± 17	47 ± 15	4	34	0.978**	0.976**	3
TS (mV)	0.93 ± 0.23	0.96 ± 0.26	16	26	0.388	0.407	0.27
CSP (ms)	184 ± 24	187 ± 25	5	13	0.799**	0.810**	15
$SICI_{2ms}$	0.31 ± 0.21	0.37 ± 0.27	42	69	0.421	0.417	0.25
$SICI_{3ms}$	0.25 ± 0.18	0.26 ± 0.14	51	65	−0.187	−0.207	0.25
ICF_{9ms}	1.16 ± 0.53	1.04 ± 0.63	34	54	0.267	0.278	0.70
ICF_{12ms}	1.10 ± 0.60	1.52 ± 0.89	44	57	0.095	0.089	1.05
$LICI_{100ms}$	0.18 ± 0.16	0.12 ± 0.11	55	93	0.217	0.209	0.17

*$p \leq 0.05$; **$p \leq 0.01$

[a]Pearson's correlation coefficient between time 1 and time 2

[b]Intra-class correlation coefficient of the absolute agreement between single measures of time 1 and time 2 using a two-way mixed model

Transcranial magnetic stimulation

After scanning individual MEP trials for outliers (± 3 SD), 1.65% of all trials were removed across all participants and TMS variables. After excluding outlier trials, the lowest number of trials used for analysis was 9. The means (M), standard deviations (SD), intra- and inter-subject coefficients of variation, test–retest correlations, ICC and RCI values for rMT, %MSO, CSP, SICI, ICF, and LICI are presented in Table 2. Overall, inter-subject COVs were larger than intra-subject COVs. Furthermore, TS-MEP were sufficiently stable across time (intra-subject COVs of 16%) for %MSO to be considered an adequate measure of corticospinal excitability. It can also be seen that, at both time points and compared to the MEP amplitude at test stimulus intensity, $SICI_{2ms}$, $SICI_{3ms}$, and $LICI_{100ms}$ reduced MEP amplitudes, and ICF_{9ms} and ICF_{12ms} increased MEP amplitudes. However, inhibitory (SICI and LICI) effects were statistically significant at the Bonferroni-corrected significance level (all $p \leq 0.05/5 = 0.01$), while facilitatory (ICF) effects were not

statistically significant (all $p \geq 0.226$) due to higher variability and smaller effect sizes at both time points.

Paired samples t tests revealed no significant effect of time for rMT ($t_{(9)} = 0.114$, $p = 0.912$), %MSO ($t_{(9)} = 0.258$, $p = 0.803$), $SICI_{2ms}$ ($t_{(9)} = 0.759$, $p = 0.467$), $SICI_{3ms}$ ($t_{(9)} = 0.167$, $p = 0.871$), ICF_{9ms} ($t_{(9)} = 0.543$, $p = 0.600$), ICF_{12ms} ($t_{(8)} = -0.940$, $p = 0.375$), $LICI_{100ms}$ ($t_{(8)} = 0.909$, $p = 0.390$), and CSP ($t_{(9)} = -0.528$, $p = 0.610$). Further reliability analyses revealed near-perfect test–retest correlations and excellent ICCs for rMT ($r_{(10)} = 0.965$, $p < 0.001$; $r_{(10)} = 0.968$, 95% CI [0.875, 0.992], $F_{(9, 9)} = 54.864$, $p < 0.001$) and %MSO ($r_{(10)} = 0.978$, $p < 0.001$; $r_{(10)} = 0.977$, 95% CI [0.9135, 0.994], $F_{(9, 9)} = 79.776$, $p < 0.001$) measurements. No significant test–retest correlations (all $p > 0.05$) and poor ICCs were found for all paired-pulse measures, except for $SICI_{2ms}$, which showed a fair, but non-significant ICC ($r_{(10)} = 0.417$, 95% CI [−0.255, 0.815], $F_{(9, 9)} = 2.372$, $p = 0.107$). CSPs showed a strong test–retest correlation ($r_{(10)} = 0.799$, $p = 0.006$) and an excellent ICC ($r_{(10)} = 0.810$, 95% CI [0.409, 0.949], $F_{(9, 9)} = 8.936$, $p = 0.002$).

Relationship between MRS and TMS measures

The systematic examination of correlations between three water-referenced MRS measures (GABA, Glx, GABA/Glx) and eight TMS measures (rMT, %MSO, CSP, and $SICI_{2ms}$, $SICI_{3ms}$, ICF_{9ms}, ICF_{12ms}, and $LICI_{100ms}$ indexes) using a Bonferroni-corrected significance level ($\alpha = 0.05/8 = 0.00625$) for multiple comparisons revealed no significant effect (all $p \geq 0.041$). The statistical values for the systematic examination of correlations between MRS and TMS measures are shown in Table 3.

Correlation analysis between the intra-subject COV of three MRS measures (COV_GABA, COV_Glx, COV_GABA/Glx) and eight TMS measures (COV_rMT, COV_%MSO, COV_CSP, and COV_$SICI_{2ms}$, COV_$SICI_{3ms}$, COV_ICF_{9ms}, COV_ICF_{12ms} and COV_$LICI_{100ms}$ indexes) using a Bonferroni-corrected significance level ($\alpha = 0.05/8 = 0.00625$) for multiple comparisons revealed no significant result. Using an uncorrected significance level ($\alpha = 0.05$), a strong positive correlation was found between the coefficient of variation of Glx and %MSO ($r_{(10)} = 0.682$, $p = 0.030$), suggesting that participants that showed greater Glx variability also showed greater variability in cortical excitability. A positive and weak, but not statistically significant ($p \geq 0.653$) correlation was found between %MSO/rMT ratios and metabolite (GABA, GABA/Glx) levels at both time points, suggesting that higher GABA levels and GABA/Glx ratios may be associated with higher intensities, relative to the rMT, needed to produce MEPs of 1 mV.

Discussion

The goal of the present study was to assess the long-term stability of TMS measures of GABA and glutamate synaptic activity and MRS measures of GABA/H_2O and Glx/H_2O concentration in sensorimotor cortex of healthy individuals. While MRS measures were stable over time, TMS measures were found to be reliable for rMT, %MSO, and CSP only. Among paired-pulse TMS measures, $SICI_{2ms}$ yielded fair, but not statistically significant reliability statistics. Additionally, correlation analysis revealed no significant relationship between TMS and MRS measures at any time point. Furthermore, stability and reliability statistics of tCr- and NAA-referenced metabolites were also obtained and found to be equivalent to or poorer than water-referenced values, which is expected as the protocol used in this study is optimized for water-referenced GABA detection. Therefore, we limit the following discussion to water-referenced spectroscopy values.

Magnetic resonance spectroscopy

Despite differences in regions of interest, between-sessions intervals, data referencing, editing and processing

Table 3 Correlation coefficients between water-referenced metabolite and TMS measures at T_1 and T_2

	Time 1			Time 2		
	GABA	Glx	GABA/Glx	GABA	Glx	GABA/Glx
RMT	−0.04	0.18	−0.14	0.11	0.54	0.01
%MSO	0.05	0.16	−0.03	0.13	0.52	0.03
%MSO/RMT	0.16	0.05	0.14	0.16	0.18	0.13
$SICI_{2ms}$	−0.26	−0.17	−0.25	−0.42	−0.65*	−0.32
$SICI_{3ms}$	−0.29	−0.37	−0.21	−0.01	0.04	−0.03
ICF_{9ms}	0.12	−0.02	0.18	−0.08	−0.65*	0.05
ICF_{12ms}	−0.29	−0.05	−0.31	0.25	−0.16	0.29
$LICI_{100ms}$	−0.09	0.08	−0.18	0.63	0.10	0.64*
CSP	0.20	0.37	0.09	−0.33	0.41	−0.42

*$p \leq 0.05$; **$p \leq 0.00625$ (Bonferroni-corrected significance level)

techniques, within-subject COVs (10%) in the present study do not diverge significantly from those reported in studies investigating the short-term reproducibility and stability of GABA and GABA + (GABA + MM), where COVs of ≈4 to 15% have been reported (Bogner et al. 2010; Evans et al. 2010; Harada et al. 2011; O'Gorman et al. 2011; Near et al. 2013, 2014; Greenhouse et al. 2016). This suggests that variations in GABA levels measured here are likely attributable to measurement error rather than long-term changes in metabolite concentration. Indeed, Evans et al. (2010) reported similar within- (8.8%) and between-subject (0.5–19.7%) single-day COVs for sensorimotor GABA/H_2O. In addition, Greenhouse et al. (2016) have found a within-subject creatine-referenced COV of 3.9 ± 1.0% and a strong test–retest correlation ($r = 0.64$) over two scans across an approximate 2-week period in the sensorimotor region, which is similar to the present findings. Furthermore, a 7-month longitudinal study reported GABA+/Cr levels with a low COV (4.3%) and a fair level of absolute agreement ($r = 0.52$) in the occipital cortex (Near et al. 2014). The excellent absolute agreement between single measurements across time ($r = 0.75$) suggests that GABA measurements in the present study are highly reliable despite COVs that are higher than those reported by Near et al. (2014) in the occipital cortex. Furthermore, ICC values for GABA are similar to that of another study, which reported a good ICC for GABA/tCr when assessing its short-term (3 h) reliability (Dyke et al. 2017).

An average within-subject COV of 4% and a fair level of absolute agreement were found for Glx, suggesting that concentrations vary little over time. Indeed, Glx COVs were consistent with those reported in reproducibility and short-term studies using different ROIs and MRS sequences (Hurd et al. 2004; Jang et al. 2005; O'Gorman et al. 2011). For example, in the dorsolateral prefrontal cortex, O'Gorman et al. (2011) reported within-session COVs of 6% between four measurements acquired within the same scanning session, while Hurd et al. (2004) reported a COV of < 10% in the parietal cortex over multiple scans. This suggests that variability estimates for Glx, as was the case for GABA, were likely due to measurement error.

The present data thus show that GABA/H_2O and Glx/H_2O concentrations in the sensorimotor cortex of healthy individuals are stable over a 3-month period. Furthermore, specialized acquisition (MEGA-PRESS) and analysis techniques (LCModel) allow stable and precise measurements of GABA and Glx at moderate field strengths (3 T) and minimize the impact of macromolecular contamination of GABA signals. In addition, no significant difference was found in GABA concentration in the sensorimotor area between older and younger adults, indicating that a significant change in individual GABA levels would not stem from aging (Mooney et al. 2017; Hermans et al. 2018). However, glutamate concentration decreases during adulthood (Grachev and Apkarian 2001). By taking into account the latter findings as well as the present results, it appears that GABA concentrations could be used as markers for monitoring disease progression and treatment effects in neurological and psychiatric populations, and Glx may also be used over shorter intervals or after taking into account the impact of aging on neurochemical concentrations. Furthermore, RCI analysis suggests that a change in water-referenced GABA levels of ≈0.15 or a change in water-referenced Glx levels of ≈0.72 across time, using the present protocol, would most likely reflect a significant alteration in metabolite concentration.

Transcranial magnetic stimulation

The excellent reliability of rMT measures found in the present study support previous reports where test–retest stability were found to be excellent within a 1-month (Hermsen et al. 2016) or 3-month period (Ngomo et al. 2012). Similarly, the intensity required to induce MEPs of 1 mV amplitude (%MSO) was also very stable across time, which is consistent with previous reports (Maeda et al. 2002; Ngomo et al. 2012; Hermsen et al. 2016). Taken together with previous studies, the present data clearly show that rMT and %MSO values reflecting cortical excitability are very stable over periods of at least 3 months. Indeed, reliable change index values indicate that a variation greater than 3% for both rMT and %MSO can be a reliable indicator of a change in excitability within the primary motor cortex.

In the present study, paired-pulse techniques were found to be highly variable between sessions and between subjects. With respect to coefficients of variation, the present findings are in general agreement with those previously reported. Indeed, between-sessions COVs were found to be lower than between-subjects COVs. Furthermore, between-sessions COVs for all ISIs were similar to those reported in previous studies, where time intervals between sessions ranged from minutes to months (Boroojerdi et al. 2000; Orth et al. 2003; Ngomo et al. 2012). However, discrepancies between the present findings and those of previous studies are found in other reliability parameters. For short-interval cortical inhibition, a fair level of reliability was observed for $SICI_{2ms}$ (ICC = 0.417) only; $SICI_{3ms}$ showed a poor reliability. The latter finding is in disagreement with previous studies where significant and excellent test–retest correlations for $SICI_{2ms}$ and $SICI_{3ms}$ (Maeda et al. 2002; Ngomo et al. 2012; Hermsen et al. 2016; Dyke et al. 2018) were reported. Furthermore, it is unclear why only $SICI_{2ms}$ was found to be reliable, as both 2 and 3 ms SICI are thought to share the same mechanism of action (Ziemann et al. 2015). Small sample size in the present study may partly explain this finding. In addition, the above discrepancy may also stem from contamination of facilitatory processes which may reduce

the net inhibitory responses following $SICI_{3ms}$ to a greater extent than $SICI_{2ms}$ (Peurala et al. 2008). With respect to intracortical facilitation, statistically non-significant and poor test–retest correlations were obtained for both ICF_{9ms} ($r = 0.267$) and ICF_{12ms} ($r = 0.379$), which is not surprising given that facilitatory protocols did not produce robust effects on MEP amplitudes in our study. This result is in partial agreement with previous findings, where test–retest correlations ranged from strong to non-existent (Maeda et al. 2002; Ngomo et al. 2012; Hermsen et al. 2016; Dyke et al. 2018). As for $LICI_{100ms}$, the obtained low reproducibility ($r = -0.052$) may be partially explained by a statistical floor effect.

The lack of consensus across studies reporting paired-pulse TMS reliability measures emphasizes the fact that care should be taken when interpreting paired-pulse measures across time or following interventions. However, discrepancies in outcome may be due to methodological differences between studies. For example, it has been shown that increasing the number of TMS-induced MEPs to at least 20 diminishes trial-to-trial variability and leads to more stable measures (Chang et al. 2016; Goldsworthy et al. 2016). It is therefore possible that collecting additional MEPs would have increased TMS sensitivity and reproducibility in the present study. Other studies have demonstrated that methodologies assessing cortical inhibition and facilitation based on threshold tracking techniques (TTT) instead of paired-pulse MEP ratios yield more stable findings (Murase et al. 2015; Mooney et al. 2017; Samusyte et al. 2018). In addition, a systematic examination of the effect of varying CS intensity in paired-pulse paradigms showed that response variability between individuals at a specific ISI was substantial across CS intensities (Orth et al. 2003). Indeed, individuals that showed strong inhibition at ISI_{2ms} at a CS intensity of 60% rMT did not necessarily show strong inhibition for the same ISI at 70% rMT. Using different %rMT as a CS would have been interesting in determining which %rMT yields more stable and reliable ppTMS measures. Furthermore, performing a similar reliability assessment using the active motor threshold (%AMT) as a basis for TMS measurement would have been of interest, since intracortical inhibition and facilitation were found to be strongly correlated to %AMT (Orth et al. 2003). Therefore, due to the great heterogeneity between paired-pulse protocols, care should be taken when comparing paired-pulse studies with different methodologies. Lastly, it must be noted that reliable change indexes ranging from 0.17 to 1.05 for paired-pulse indexes were obtained in the present study, which may be appropriate for clinical purposes. However, the absence of statistical significance for reliability statistics used in calculating ppTMS RCIs suggests that those should be used carefully.

Finally, for CSP measurements, a previous study (Hermsen et al. 2016) reported that the reliability of CSP length, which was measured visually or automatically, yielded similar test–retest correlation coefficients (visual $r = 0.466$; automated $r = 0.486$). The results of the present work support these findings, as an excellent absolute agreement was found across time ($r = 0.81$) when CSP length was examined visually. Furthermore, both intra- (COV = 5%) and inter-subject (COV = 13%) variability were found to be low. Thus, CSP length seems to be stable across time over a minimal time interval of 3 months. As a result, the reliable change index can be duly interpreted and suggests that an inter-session variation of at least 15 ms is significant.

MRS and TMS

In the present study, no correlations were found between MRS and TMS measures at either time point, giving further credence to the idea that MRS and TMS have different neurochemical substrates. Furthermore, exploratory analyses revealed no significant relationship between intra-subject variability of TMS and MRS measures.

Transcranial magnetic stimulation is believed to mainly reflect receptor-dependent activity. Indeed, it has been shown that that MT is a measure of corticospinal excitability and is thought to depend on glutamatergic synaptic activity (Hodgkin and Huxley 1952; Paulus et al. 2008). Furthermore, SICI appears to rely on fast-acting $GABA_A$ receptor-mediated inhibition (Di Lazzaro et al. 1998), while LICI involves slow-acting $GABA_B$ receptor-mediated inhibition (Ziemann et al. 2015) and ICF is believed to implicate both glutamatergic and GABAergic receptor networks (Ziemann et al. 2015). While it is believed that spinal mechanisms contribute to the early part of CSP (the first 50–75 ms), its late part is thought to reflect motor cortical postsynaptic inhibition ($GABA_AR$ and $GABA_BR$ activity) (Fuhr et al. 1991; Inghilleri et al. 1993; Ziemann et al. 1993, 2015).

Magnetic resonance spectroscopy, on the other hand, quantifies total neurometabolite concentrations within an area of interest and does not represent receptor activity. It is believed that MRS-GABA mainly reflects extrasynaptic concentrations (Rae 2014; Stagg 2014; Dyke et al. 2017). Extrasynaptic GABA is thought to mediate tonic inhibition and is involved in regulating tonic and phasic activity in GABAergic circuits (Wu et al. 2007; Glykys et al. 2008). In a similar manner to GABA, MRS-Glx also measures total Glx concentration in a given area. However, ambiguity remains as to the precise substrates of MRS-Glx, which combines two signals stemming from Glu and Gln that cannot be resolved using MEGA-PRESS at 3 T. Furthermore, since these two neurometabolites are involved in different neurobiological processes and constantly undergo dynamic exchange through the Glu/Gln cycle (Bak et al. 2006; McKenna 2007), it is difficult to precisely pinpoint what comprises MRS-Glx, and how it relates to neurophysiological functioning.

Due to their very different modes of action, it is not surprising that receptor activity-dependent TMS measures do not to correlate with MRS-GABA and MRS-Glx. Indeed, no correlations were found between MRS-GABA and MRS-Glx (or MRS-Glu) concentrations and TMS measures of cortical inhibition or facilitation in previous studies (Stagg et al. 2011a; Tremblay et al. 2013; Dyke et al. 2017; Hermans et al. 2018). However, Tremblay et al. (2013) reported a significant correlation between MRS-Glx and cortical silent period length, which was not replicated in the present study. The present results are, however, in agreement with the suggested mechanism of action underlying CSP duration, which is thought to be glutamate independent (Ziemann et al. 2015).

The absence of significant correlations between MRS-GABA and TMS measures thus appear to be replicated across MR sequences, field strength and sample size, but also across TMS techniques, giving further credence to the idea that MRS measures of GABA do not reflect TMS-derived measures of cortical inhibition or facilitation. Furthermore, given that GABA levels were found to be similar in both young and older adults, while some TMS measures of cortical excitability and inhibition were found to be modulated by age (Mooney et al. 2017; Hermans et al. 2018), it is likely that both techniques possess different neurochemical substrates, with respect to GABA and its associated receptors. The lack of correlation between MRS-Glu (or MRS-Glx) and TMS measures of cortical inhibition, facilitation and silent period has also been replicated across studies (Stagg et al. 2011b; Tremblay et al. 2013; Dyke et al. 2017). However, the relationship between global cortical excitability and MRS-Glu remains ambiguous. Indeed, previous studies have reported conflicting results with respect to the relationship between MRS-glutamate and the slope of the input/output curve, which indexes global corticospinal activity (Stagg et al. 2011a; Dyke et al. 2017). Another study has found a positive correlation between MEP amplitudes and motor cortical GABA/Cr concentrations, which was not replicated in the present study (Greenhouse et al. 2017). Finally, the present study showed that the intraindividual variability of TMS measures does not appear to be predictive of intraindividual variability of MRS measures. Indeed, no statistically significant correlation between MRS-COVs and TMS-COVs was found for any measurement. This suggests that TMS and MRS variations in measurement stability are independent of each other, further strengthening the argument that distinct inhibitory/excitatory mechanisms can be assessed by the two techniques.

Conclusion

This study revealed that water-referenced MRS-GABA and MRS-Glx have good stability over a 3-month period, with variability across time comparable to that of other studies where measurements were taken at different time intervals and in different brain areas. (Near et al. 2014; Dyke et al. 2017). While rMT, %MSO, and CSP were found to be stable over time, paired-pulse TMS measures showed greater variability and lesser reliability. Therefore, MRS (GABA, Glx) and some TMS (rMT, %MSO, CSP) measures possess robust methodological properties that make them reliable markers of disease progression and treatment effects. The present study also added to the existing literature suggesting that MRS and TMS measures do not reflect the same neurochemical events, while showing for the first time that the long-term stability of the two techniques are independent of each other.

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Compliance with ethical standards

Conflict of interest The authors report no conflicts of interest.

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