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Phase-adjusted echo time (PATE)-averaging ^1H MRS: application for improved glutamine quantification at 2.89 T

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Abstract

^1H MRS investigations have reported altered glutamatergic neurotransmission in a variety of psychiatric disorders. The unraveling of glutamate from glutamine resonances is crucial for the interpretation of these observations, although this remains a challenge at clinical static magnetic field strengths. Glutamate resolution can be improved through an approach known as echo time (TE) averaging, which involves the acquisition and subsequent averaging of multiple TE steps. The process of TE averaging retains the central component of the glutamate methylene multiplet at 2.35 ppm, with the simultaneous attenuation of overlapping phase-modulated coupled resonances of glutamine and *N*-acetylaspartate. We have developed a novel post-processing approach, termed phase-adjusted echo time (PATE) averaging, for the retrieval of glutamine signals from a TE-averaged ^1H MRS dataset. The method works by the application of an optimal TE-specific phase term, which is derived from spectral simulation, prior to averaging over TE space. The simulation procedures and preliminary *in vivo* spectra acquired from the human frontal lobe at 2.89 T are presented. Three metabolite normalization schemes were developed to evaluate the frontal lobe test–retest reliability for glutamine measurement in six subjects, and the resulting values were comparable with previous reports for within-subject (9–14%) and inter-subject (14–20%) measures. Using the acquisition parameters and TE range described, glutamine quantification is possible in approximately 10 min. The post-processing methods described can also be applied retrospectively to extract glutamine and glutamate levels from previously acquired TE-averaged ^1H MRS datasets.

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Keywords

^1H MRS; glutamine; glutamate; TE averaging; phase-adjusted TE averaging

INTRODUCTION

Evidence from clinical ^1H MRS investigations performed in human subjects has implicated abnormal glutamatergic neurotransmission in a wide range of psychiatric illnesses, including schizophrenia (1,2), bipolar disorder (3–6), depression (7,8), anxiety disorders (9), substance abuse (10,11) and conditions characterized by neurocognitive deficits (12). For such studies, the reliable separation of glutamate (Glu) from glutamine (Gln) is of critical importance, as glutamatergic dysfunction may be associated with altered Glu and/or Gln concentration (13). However, the resolution of Glu from Gln is often hampered by the severe peak overlap of their scalar spin–spin (J)-coupled resonances, which is a direct consequence of their closely similar molecular structures. Glu and Gln resonance overlap is particularly problematic for ^1H MRS studies performed at clinical static magnetic field strengths (B_0 3.0 T), and their separation is made worse by the strong signal overlap with the J -coupled methylene resonances of *N*-acetylaspartate (NAA).

Accordingly, a number of ^1H MRS methods have been proposed to improve Glu and Gln separation at low to medium B_0 field strengths (1.5–4.0 T). Perhaps the simplest approaches have involved the optimization of pulse sequence timing intervals, such as the echo time (TE) in point-resolved spectroscopy (PRESS) (14) and TE and mixing time (TM) in stimulated echo acquisition mode (STEAM) (15,16) sequences. More sophisticated ^1H MRS approaches have demonstrated Glu/Gln resolution using multiple quantum filtration (17), J -refocusing and coherence transfer methods (18,19), chemical shift-selective filters (20), exploitation of chemical shift displacement (21), multiple spin refocusing (22) and spectrally selective spin refocusing (23). Glu resolution can also be improved through the use of two-dimensional (2D) ^1H MRS techniques, including homonuclear 2D correlated spectroscopy (24), constant-time PRESS (25) and 2D J -resolved ^1H MRS with prior knowledge fitting (26). Directly related to 2D J -resolved ^1H MRS is the TE-averaging method for Glu detection (27), which involves the averaging of multiple spectra acquired with a range of incrementally stepped TEs. The process of TE averaging retains the central component of the Glu methylene (C4) multiplet at 2.35 ppm, with the simultaneous attenuation of overlapping phase-modulated methylene resonances of Gln and NAA. Implementation of the TE-averaging ^1H MRS method and the spectral fitting of the simplified Glu C4 proton lineshape (often referred to as a pseudo-singlet) are relatively straightforward. As such, the TE-averaging ^1H MRS technique has found clinical application for Glu measurement in several human disease states (11,12,28,29).

A previous report has demonstrated the signal recovery of the Gln C4 proton resonance from TE-averaged ^1H MR spectra acquired *in vivo* (30). Using spectral simulation procedures, this study investigated the application of a step function filter where, instead of additive averaging, data were subtracted after a certain TE point. This resulted in the constructive averaging of the Gln C4 proton peak with destructive attenuation of the Glu C4 resonance.

Here, we introduce an alternative and novel post-processing method for the retrieval of Gln resonances from a TE-averaged ^1H MRS dataset, which we have termed phase-adjusted echo time (PATE)-averaged ^1H MRS. The PATE-averaging ^1H MRS technique applies an optimal zero-order phase term, determined using spectral simulations, to maximize the Gln C4 proton signal integral prior to TE averaging. This article describes the PATE-averaging MRS simulation procedures and preliminary data acquired from the human frontal lobe to demonstrate the potential clinical utility of the technique. The *in vivo* test–retest reliability was evaluated using three different metabolite normalization schemes.

METHODS

Simulation procedures

PRESS ^1H MR spectral simulations were performed in MATLAB R2011b (The Mathworks, Natick, MA, USA) in conjunction with the MATLAB Parallel Computing™ toolbox utilizing four dual-quad 2.8-GHz Intel® xenon processors. For all metabolites, the three-dimensional (3D) localized simulations employed procedures described in previous reports (31,32), with product-operator transformation matrices initially being calculated for each slice selection step using a 1% spatial resolution within the slice profile. Hence, the 3D simulations allowed for the incorporation of the experimental radiofrequency (RF; see ‘Data acquisition’ section) and gradient pulses in addition to Zeeman, chemical shift and J -coupling effects for each metabolite. Spectra were calculated for a static magnetic field strength (B_0) of 2.89 T corresponding to a ^1H MR frequency of approximately 123 MHz. A total of 48 ^1H MR spectra were simulated for choline (Cho), creatine (Cre), Gln, Glu, γ -aminobutyric acid (GABA), *myo*-inositol (Ins) and NAA, spanning a TE range of 30–500 ms. The Cre and NAA methylene (CH_2) and methyl (CH_3) proton groups were generated independently. All metabolite chemical shift values and J -coupling constants were taken from the literature (33). The initial PRESS TE period (TE1) was fixed at 12 ms, with the second TE period (TE2) incremented to realize the required total TE. All spectra were line broadened to a realistic *in vivo* linewidth (exponential filter, 6 Hz) and subjected to signal amplitude weighting in order to reflect the physiologic concentration ratios [Glu]: [Gln]: [GABA]: [Ins]: [Cho]: [Cre]: [NAA] = 8: 4: 1: 5: 2: 8: 10. Using recent literature values (34), metabolite-specific spin–spin (T_2) relaxation time filters were applied along the TE dimension with the Gln and GABA T_2 relaxation times assumed to match that of Glu. ^1H group-specific T_2 values were also applied for both the Cre and NAA CH_2 and CH_3 protons (34). Subsequently, a zero-order phase loop ($-\pi$ to π radians) was applied to all 48 TE-stepped Gln spectra and, for each phase step, Gln peak integration was performed between the 2.3–2.6-ppm chemical shift region. The optimal TE-specific zero-order phase $\phi_0(\text{opt})$ yielded the maximum Gln signal area. Simulated PATE-averaged ^1H MR spectra were reconstructed for all seven metabolites by applying $\phi_0(\text{opt})$ prior to averaging over TE space.

Human subject measurements

Data acquisition—Six healthy control subjects [two females; mean age $\pm$ standard deviation (SD), 24 ± 3 years] were scanned on two consecutive days. All *in vivo* measurements were performed using a 2.89-T Siemens (Erlangen, Germany) MAGNETOM

Trio™ whole-body MRI/MRS system controlled using the *syngo* software version ‘VB17’. A circularly polarized body coil was used for RF transmission with a 12-channel phased-array receive-only head coil used for signal reception. Subjects were positioned supine within the head coil and additional foam pads were arranged in an effort to optimally fixate the subject’s head. The head coil was used in a four-cluster configuration, with data received from two anteriorly oriented and two posteriorly oriented coil clusters. High-resolution magnetization-prepared rapid gradient echo (MP-RAGE) MR images (TR/TE/TI = 2000/3.53/1100 ms; field of view, $256 \times 256 \times 224$ mm³; isotropic resolution, 1 mm) were acquired initially to facilitate voxel positioning and for brain tissue segmentation. The image data were used to position an oblique $3.0 \times 3.0 \times 2.5$ -cm³ voxel bilaterally within the anterior cingulate cortex (ACC) with localized shimming subsequently performed to obtain unsuppressed water signal linewidths of 9 Hz or less. Standard PRESS ¹H MR spectra [TR/TE = 2000/30 ms; number of excitations (NEX) = 128] and PRESS ¹H MR spectra with multiple TE steps (TR/TE = 2000/30–500 ms; TE = 10 ms; NEX per TE = 8) were acquired from all subjects at both time points. PRESS localization employed a Hanning-filtered sinc RF pulse of 2.6 ms duration (bandwidth, 5 kHz) for slice-selective excitation, followed by two identical optimized sinc RF pulses (35) of 7.0 ms duration (bandwidth, 1 kHz) for slice-selective refocusing. All PRESS spectra were acquired with TE1 fixed and set to 12 ms, thus matching the simulation timing parameters. In addition, for all TE steps, PRESS spectra were acquired without water suppression (TR = 2000 ms; NEX = 4). Outer-volume suppression was used in all PRESS measurements, achieved with six saturation bands excited using hyperbolic secant adiabatic full-passage RF pulses.

Tissue segmentation—Brain extraction and tissue-type segmentation was applied to all MP-RAGE image data using the BET (36) and FAST (37) tools provided with the freely available FMRIB software library (38). Home-written MATLAB functions were then used to extract the 3D volume corresponding to the positioned MRS voxel and to calculate the within-voxel gray matter (GM), white matter (WM) and cerebrospinal fluid (CSF) tissue content for each subject. The within-voxel GM fraction was calculated as the ratio to total brain matter, i.e. $100 \times \text{GM}/(\text{WM} + \text{GM})$.

Data processing—All free induction decay (FID) data were collected individually without signal averaging and saved as Siemens TWIX files. The spectral data preprocessing steps described hereafter were performed using home-written MATLAB functions. First, the individual FIDs recorded at each cluster were signal averaged for each TE step to yield four (PRESS; 4×1 TE step) or 192 (multi-TE PRESS; 4×48 TE steps) separate water-suppressed FIDs. The individual FID data were then subjected to time domain eddy current correction (39) using the corresponding cluster and TE-specific unsuppressed water signal. The TWIX file header contains individual coil cluster-specific weighting function coefficients, and these were applied to the metabolite and unsuppressed water FIDs prior to the combination of equivalent TE data across the four clusters. These preprocessing steps yielded a single FID for PRESS and 48 FIDs for multi-TE PRESS. Standard TE averaging was carried out as described in ref. (27), whereas PATE averaging applied the simulation-derived TE-specific $\varphi_0(\text{opt})$ prior to averaging over TE space.

Spectral quantification—PRESS, TE-averaged and PATE-averaged ^1H MRS data were fitted using the commercially available Linear Combination (LC) Model software (40; version 6.1.4E) in conjunction with an appropriate simulated basis set. The individual metabolite basis functions for each data type were generated using identical procedures to those described in the ‘Simulation procedures’ section. In addition to Glu, Gln, GABA, Ins, Cho, Cre and NAA, additional basis functions were generated for alanine (Ala), aspartate (Asp), lactate (Lac), *N*-acetylaspartyl Glu (NAAG), *scyllo*-inositol (sI) and taurine (Tau). For each data type, the basis function used for LC Model fitting of NAA was the linear sum of the relevant T_2 -corrected NAA CH_2 and CH_3 simulations. The LC Model fit analysis window was set to cover the 0.5–4.5-ppm chemical shift region.

Three different metabolite peak normalization schemes were evaluated as follows. The first approach normalized the LC Model-reported metabolite peak area to the PRESS (TE = 30 ms) unsuppressed water signal integral, which was calculated after fitting a Voigt lineshape to the real component of the phased frequency domain unsuppressed water data (41). The nonlinear least-squares ‘lsqnonlin’ function provided with the MATLAB Optimization Toolbox™ was used to fit the water data, with the initial estimate for signal amplitude being subject specific and based on the maximum peak amplitude. An initial estimate of 8 Hz was used for the signal linewidth with the lower and upper bounds set to 1 and 20 Hz, respectively. The resulting metabolite/water ratios were corrected for the within-voxel CSF fraction determined from the segmented MRI data. The second method normalized the metabolite peak area to the Cre 3.0-ppm CH_3 integral. This approach initially involved the simulation of TE-averaged and PATE-averaged ^1H MRS datasets that consisted of all 13 metabolites in equal concentrations. Subsequently, after determining the LC Model metabolite/Cre ratios for the simulated data, *in vivo* concentration estimates were calculated for Gln, Glu and NAA, assuming a Cre concentration of 8 mM. The third approach calculated Gln/Glu ratios using the Gln and Glu peak integrals extracted from PATE-averaged and TE-averaged MRS datasets, respectively. Within-subject test–retest measurement reliability was evaluated for each quantification scheme using the coefficient of variation (CV), expressed as a percentage of SD divided by the mean of the two scans. Inter-subject CVs for Gln were also calculated for each quantification scheme.

RESULTS

Figure 1a shows the simulation-derived $\phi_0(\text{opt})$ for Gln plotted as a function of TE. The calculated Gln peak area measured between 2.3 and 2.6 ppm is then plotted for all 48 TE steps for the $\phi_0(\text{opt})$ phase-adjusted and phase-unadjusted conditions in Fig. 1b. The positive signal area at each of the 48 TE steps in the phase-adjusted data should be noted. Figure 1c shows a series of selected spectra (TE = 170–200 ms), illustrating the simulated Gln multiplet lineshape before and after the application of $\phi_0(\text{opt})$. The Gln $\phi_0(\text{opt})$ determined from the simulation was applied subsequently to Glu, GABA and NAA for each TE step. Figure 2 displays the resulting relative signal areas plotted for all four metabolites, which provided a visual basis and starting point for the empirical determination of an optimal TE range for PATE averaging. Briefly, blocks of contiguous TE steps were systematically averaged until the Gln signal area was maximized through constructive averaging with the most favorable minimization of overlapping Glu and NAA multiplets. The optimal TE range

was found to consist of 19 of the 48 TE steps (50–230 ms), which is the TE range used hereafter for both TE- and PATE-averaging spectral reconstructions.

Figure 3 displays simulated PRESS, TE-averaged and PATE-averaged ^1H MR spectra for Glu, Gln, GABA, Ins, Cho, Cre and NAA. The summed ‘composite’ spectra with and without Gln are also presented for each data type. The simulated short-TE PRESS data clearly illustrate the problematic and severe peak overlap of the Gln C4 proton resonance with the Glu C4 and NAA methylene multiplets. The simulated TE-averaged spectrum shows a well-resolved Glu C4 proton peak at 2.35 ppm with an attenuated Gln C4 proton peak that is largely cancelled out by an inverted component of the NAA methylene proton multiplet. Conversely, the simulated PATE-averaged MRS data show a Gln C4 multiplet of comparable amplitude to the Glu C4 peak and with larger amplitude than the coincident NAA methylene multiplet. Signal overlap of Gln and GABA resonances is fairly negligible for each data type. These observations from simulation are summarized quantitatively in Table 1, which presents the Gln/Glu, Gln/GABA and Gln/NAA signal integral ratios measured between 2.3 and 2.6 ppm for each of the three data types.

Figure 4 displays tissue-segmented axial and sagittal slices extracted from a 3D MP-RAGE dataset recorded from a single subject. The typical MRS voxel position is also depicted in Fig. 4. The mean within-voxel GM content was calculated as $70 \pm 4\%$ (mean $\pm$ SD), whereas the within-subject CV for the GM content was 2%. Figure 5 shows the PATE-averaged ^1H MR spectrum recorded from the voxel presented in Fig. 4, illustrating LC Model fits for seven metabolites, as well as the baseline fit and residuum. Table 2 reports the water-normalized within-subject Gln, Glu and NAA CV values and LC Model-derived Cramer–Rao lower bound (CRLB) values for each of the three data types. The concentration estimates and observed within-subject CVs based on Cre normalization are summarized in Table 3 for Gln, Glu and NAA. The third quantification method, which calculated Gln/Glu ratios, showed a mean within-subject CV of 14%. Mean inter-subject CV values of 14%, 20% and 26% were calculated for Gln measurements using the water normalization-, Cre normalization- and Gln/Glu-based quantification methods, respectively.

DISCUSSION

The novel post-processing method introduced in this article, termed PATE-averaged ^1H MRS, may improve the differentiation of Gln and Glu, as demonstrated for a B_0 field strength of 2.89 T. The method utilizes datasets acquired with multiple TE steps (e.g. TE-averaged or 2D J -resolved ^1H MRS data) and applies an optimal simulation-derived TE-specific phasing parameter prior to averaging across TE space. The objective of the phasing parameter is to maximize the peak integral of the target Gln multiplet prior to TE averaging. Ideally, the reduction of the overlapping multiplets from Glu and NAA resonances should be attenuated during these procedures. The resulting simulations show a positive signal integral for Gln between 2.3 and 2.6 ppm for each TE step, whereas the calculated peak area from the overlapping multiplets of GABA, Glu and NAA fluctuates from positive to negative values. Importantly, the simulations show that the Gln/Glu and Gln/NAA signal area ratios calculated between 2.3 and 2.6 ppm increase by approximately twofold for PATE-averaged ^1H MRS relative to standard PRESS.

The *in vivo* utility and test–retest reliability of the PATE-averaged ^1H MRS approach were demonstrated using data acquired from the ACC of six subjects in different scan sessions. The ACC was chosen as this is a brain region with functional relevance and is thought to be a primary locus in many psychiatric disorders. LC Model and simulated basis sets were used for prior knowledge fitting, and expressed as CSF-corrected water-normalized levels, Crereferenced concentration estimates and Gln/Glu ratios. Using these normalization approaches, we calculated mean ($\pm$ SD) within-subject Gln CV values ranging between 9% and 14%, and these values should be placed in context with similar values documented for a comparable B_0 field strength. Mullins *et al.* (42) demonstrated the use of a PRESS sequence with an optimized TE (40 ms) that improved the measurement reliability of several J -coupled metabolite resonances, including Gln. The same session within-subject CRLB and CV values provided in that study were 29% and 37%, respectively, although we acknowledge that, for direct comparison, our CV values should be multiplied by 2 (i.e. 13%) to be consistent with their method of CV calculation. Similarly, in a separate report, quantification of 2D J -resolved ^1H MRS data acquired at 3.0 T yielded a within-subject frontal lobe Gln measurement reliability of 19%, after 11 measurements in different scan sessions from a single subject (26). Using the three different normalization methods, we calculated an inter-subject CV range of 14–26% for frontal lobe Gln levels using the PATE-averaged ^1H MRS approach, and this value can be compared with previous literature findings using STEAM (24%; 43), PRESS (31%; 44) and spectrally selective refocusing (22%; 23) methods. The authors recognize, however, that our voxel was generally larger than that used in previous studies, which would act to increase the metabolite peak signal-to-noise ratio and potentially to enhance our test–retest fit reliability. Future studies should aim to compare directly PATE-averaged ^1H MRS reliability against existing methods with equivalent voxel sizes and comparable measurement times. As a final point to this section, the Crereferenced metabolite levels in the present study were based on either TE-averaged (NAA and Glu) or PATE-averaged (Gln) ^1H MRS data, from which we estimated concentrations of 5 ± 1 , 11 ± 1 and 12 ± 2 mM for Gln, Glu and NAA, respectively. In general, these metabolite levels fit well within the range of published findings reported for healthy volunteers [(32) and citations therein].

Cytosolic macromolecule (MM) resonances and their potential contribution to the reconstructed *in vivo* PATE-averaged ^1H MRS data are discussed here. Using the total of 12 multi-TE ^1H MRS datasets acquired from the six subjects in the present study, we estimated a mean T_2 relaxation time of 23 ms for the 0.9-ppm macromolecule (MM1) resonance (data not shown). A previous study performed at 4.0 T reported MM1 T_2 relaxation times of comparable magnitude (45). The TE at mean signal intensity during a given T_2 decay, i.e. the effective TE, must be considered when averaging over multiple TEs (17). The present PATE-averaged ^1H MRS study used a TE range of 50–230 ms, resulting in an effective TE of around 94 ms for the MM1 resonance. Only 2% of the original MM1 transverse magnetization is detectable at this effective TE, such that the MM contribution to the reconstructed PATE-averaged ^1H MRS data is likely to be negligible. This is reflected in the relatively flat spectral baseline observed in the *in vivo* PATE-averaged ^1H MRS data. Nevertheless, the exact appearance of the MM resonances in the PATE-averaged data is difficult to predict through simulation procedures, and we are planning to acquire

metabolite-nulled PATE-averaged ^1H MRS data to further evaluate the magnitude of the MM spectral contribution.

The processing of TE-averaged ^1H MRS basis sets typically is performed without considering T_2 relaxation effects along the TE dimension, which can lead to the inclusion of inaccurate model spectra as a priori information. To our knowledge, a single study has accounted for metabolite-specific T_2 relaxation for the processing of TE-averaged ^1H MRS data (20). We further investigated how the incorporation of metabolite T_2 relaxation time information might affect PATE-averaged ^1H MRS and its spectral fitting by generating an additional basis set without T_2 weighting. This basis set was used to fit the identical *in vivo* dataset, and its performance was evaluated using water signal normalization. Without T_2 correction, one of the 12 spectra showed a no-fit for Gln and the mean CRLB increased from 9% to 11%. In addition, within-subject and inter-subject CV values increased from 9% to 21% and 14% to 23%, respectively, further demonstrating the requirement of incorporating basis set T_2 correction into TE-averaging fitting schemes. Directly following on from these observations, an interesting future development might investigate the use of subject-specific T_2 relaxation times for individual determination of optimal PATE-averaged ^1H MRS phasing parameters, and subsequent quantification using dedicated basis sets.

CONCLUSION

In summary, these findings suggest that the processing of TE-averaged (or 2D *J*-resolved) datasets using PATE-averaging approaches may help to improve Gln quantification whilst maintaining the option of reasonable Glu measurement reliability through conventional TE averaging. Importantly, the PATE-averaged processing methods can be applied retrospectively to previously acquired TE-averaged ^1H MRS datasets. From an acquisition standpoint, Gln resolution is attainable in approximately 10 min using the TE sampling parameters outlined here (TE = 50–230 ms). The *in vivo* data presented, recorded from a brain region with functional relevance in a wide range of psychiatric disorders, showed close similarity with simulated data and a test–retest reliability comparable with existing MRS methodologies. The exact TE range used was determined empirically from simulated data, although, in future, automated procedures should be investigated to find optimal solutions for Gln resolution. Currently, PATE-averaged ^1H MRS approaches are under investigation for the targeting of other metabolites.

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Abbreviations used

ACC   anterior cingulate cortex

Ala	alanine
Asp	aspartate
Cho	choline
Cre	creatine
CRLB	Cramer–Rao lower bound
CSF	cerebrospinal fluid
CV	coefficient of variation
2D/3D	two-dimensional/three-dimensional
FID	free induction decay
GABA	γ -aminobutyric acid
Gln	glutamine
Glu	glutamate
GM	gray matter
Ins	myo-inositol
Lac	lactate
MM	macromolecule
MP-RAGE	magnetization-prepared rapid gradient echo
NAA	N-acetylaspartate
NAAG	N-acetylaspartyl Glu
NEX	number of excitations
PATE	phase-adjusted echo time
PRESS	point-resolved spectroscopy
RF	radiofrequency
SD	standard deviation
sI	scyllo-inositol
STEAM	stimulated echo acquisition mode
Tau	taurine
TE	echo time
TM	mixing time
WM	white matter

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Figure 1.

(a) $\phi_0(\text{opt})$ for glutamine (Gln) plotted as a function of echo time (TE). (b) Gln multiplet peak integral measured between 2.3 and 2.6 ppm for phase-adjusted and phase-unadjusted conditions (au, arbitrary unit). (c) Gln spectral appearance for a series of selected spectra prior to and following the application of $\phi_0(\text{opt})$. The dotted lines represent the integrated chemical shift region used to determine $\phi_0(\text{opt})$.

Figure 2. Relative signal areas measured for glutamine (Gln), glutamate (Glu), γ -aminobutyric acid (GABA) and *N*-acetylaspartate (NAA), following the application of the simulation-derived TE-specific $\phi_0(\text{opt})$. The signal integration was performed between the 2.3–2.6-ppm chemical shift region for all four metabolites (au, arbitrary unit).

Figure 3.

Simulated point-resolved spectroscopy (PRESS), echo time (TE)-averaged and phase-adjusted echo time (PATE)-averaged ^1H MR spectra for glutamate (Glu), glutamine (Gln), γ -aminobutyric acid (GABA), *myo*-inositol (Ins), choline (Cho), creatine (Cre) and *N*-acetylaspartate (NAA). The TE-averaged and PATE-averaged ^1H MRS datasets were reconstructed using the TE range 50–230 ms. The resulting composite spectra are presented for each data type with (black spectra) and without (red spectra) the addition of the Gln component. The relative vertical scaling used to present each plot is also provided.

Figure 4.

Tissue-segmented mid-sagittal (a) and axial (b) slices extracted from a three-dimensional magnetization-prepared rapid gradient echo (MP-RAGE) dataset recorded from a 29-year-old man. White matter (WM), gray matter (GM) and cerebrospinal fluid (CSF) are represented by white, light gray and dark gray pixels, respectively. The black rectangle depicts the typical positioning of the MRS voxel within the anterior cingulate cortex (ACC), which was positioned oblique along the sagittal dimension.

Figure 5.

The LC Model-fitted phase-adjusted echo time (PATE)-averaged ^1H MR spectrum recorded from the anterior cingulate cortex (ACC) voxel shown in Fig. 4. The raw data, spectral fit and fitted residual (raw data – fit) are presented, together with the individual fits for seven metabolites. The LC Model-fitted baseline has been removed from the individual metabolite fits to enable a clearer comparison with the simulated PATE-averaged ^1H MRS data

presented in Fig. 3. Cho, choline; Cre, creatine; Gln, glutamine; Glu, glutamate; Ins, *myo*-inositol; NAA, *N*-acetylaspartate.

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Table 1

Glutamine (Gln) peak area measured between 2.3 and 2.6 ppm and normalized to the corresponding glutamate (Glu), γ -aminobutyric acid (GABA) and *N*-acetylaspartate (NAA) peak area for each data type

Data type	Gln/Glu	Gln/GABA	Gln/NAA
PRESS	0.5	7.7	0.8
TE-averaged	0.5	3.7	-1.0
PATE-averaged	1.0	9.3	2.7

PATE, phase-adjusted echo time; PRESS, point-resolved spectroscopy; TE, echo time.

Point-resolved spectroscopy (PRESS), echo time (TE)-averaged and phase-adjusted echo time (PATE)-averaged ¹H MRS test-retest reliability measures calculated using cerebrospinal fluid (CSF)-corrected tissue water integral as the normalization reference. The mean Cramer-Rao lower bound (CRLB) values and within-subject coefficients of variation (CVs) are provided for glutamine (Gln), glutamate (Glu) and *N*-acetylaspartate (NAA)

Table 2

Metabolite	PRESS		TE-averaged		PATE-averaged	
	CRLB	CV	CRLB	CV	CRLB	CV
Gln	<i>a</i>	<i>a</i>	12%	20%	9%	9%
Glu	5%	9%	4%	6%	9%	18%
NAA	2%	7%	2%	10%	3%	11%

^a Gln was successfully fitted in only one of the two PRESS spectra recorded from each subject.

Table 3

Concentration estimates and corresponding coefficients of variation (CVs) based on the use of creatine (Cre) as the normalization reference

Metabolite	Mean concentration $\pm$ SD (mM)	CV (%)
Gln ^a	5 $\pm$ 1	9
Glu ^b	11 $\pm$ 1	9
NAA ^b	12 $\pm$ 2	10

Gln, glutamine; Glu, glutamate; NAA, *N*-acetylaspartate.

calculated using the phase-adjusted echo time (PATE)-averaged^a and echo time (TE)-averaged^b ¹H MRS approaches