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# Optimized MEGA-SPECIAL for *in vivo* glutamine detection in the rat brain at 14.1 T

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**Glutamine has multiple roles in brain metabolism and its concentration can be altered in various pathological conditions. An accurate knowledge of its concentration is therefore highly desirable to monitor and study several brain disorders *in vivo*. However, in recent years, several MRS studies have reported conflicting glutamine concentrations in the human brain. A recent hypothesis for explaining these discrepancies is that a short $T_2$ component of the glutamine signal may impact on its quantification at long echo times.**

**The present study therefore aimed to investigate the impact of acquisition parameters on the quantified glutamine concentration using two different acquisition techniques, SPECIAL at ultra-short echo time and MEGA-SPECIAL at moderate echo time. For this purpose, MEGA-SPECIAL was optimized for the first time for glutamine detection.**

**Based on the very good agreement of the glutamine concentration obtained between the two measurements, it was concluded that no impact of a short $T_2$ component of the glutamine signal was detected. Copyright © 2014 John Wiley & Sons, Ltd.**

**Keywords:** MEGA-SPECIAL; glutamine; *J*-difference editing; magnetic resonance spectroscopy; transverse relaxation

## INTRODUCTION

Glutamine (Gln) is an essential amino acid in the brain, whose concentration is affected in several brain disorders such as hepatic encephalopathy (1,2), a neurological complication associated with acute and chronic liver diseases. An accurate knowledge of the Gln concentration in the healthy brain is therefore necessary for an early detection of abnormal Gln levels. However, in recent years, several studies have reported conflicting Gln concentrations in the human brain. On the one hand, *in vitro* studies have reported Gln levels of about 4 μmol/g for several brain regions (3,4), which is in agreement with *in vivo* measurements of Gln made using $^{13}$C MRS (5). On the other hand, $^{1}$H MRS studies in the human brain reported widely varying results, including Gln concentrations in a range between 1 and 4 μmol/g of wet tissue (6–13).

In an attempt to reconcile these contradictory results, a recent study performed at a magnetic field strength of 3 T simulated the spectral output of several localized $^{1}$H MRS techniques in order to evaluate the possible bias of their Gln measurement (7). However, these simulations failed to explain the discrepancy between previous studies (6–13). At the same time, the *in vivo* results of the study obtained at relatively long echo times consistently gave low Gln concentrations of about 1 μmol/g. The authors therefore suggested the possibility of an underestimation of the Gln concentration at longer echo times due to a short $T_2$ component. This hypothesis was supported by their own results and those of previous $^{1}$H MRS studies that reported a tendency to measure lower Gln concentrations when using longer echo times (TE > 30 ms) (9–11) in comparison to shorter echo time measurements (6,12). Indeed, metabolite quantification of spectra obtained at longer echo times is usually performed by using either metabolite simulations corrected with measured *in vitro* or *in vivo* $T_2$ relaxation times (generally assuming that Gln had a similar $T_2$ relaxation value to other known metabolites), or by using a basis set of measured *in vitro* metabolite spectra in similar conditions. In either case, Gln quantifications assume a single $T_2$ component in the signal decay, which can potentially lead to the acquisition of a smaller signal than expected at long echo times if a short $T_2$ component is present. However, results from previous studies have not all proved to be in agreement with the hypothesis of a short $T_2$ component, such as the high Gln concentrations obtained by Prescot *et al.* (14) at long echo time in the human brain.

Therefore, the aim of the present study was to investigate the impact of acquisition parameters on the measured Gln concentration

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**Abbreviations used:** *TE, echo time; TI, inversion time; TR, repetition time; $n_t$, number of transients; VOI, volume of interest; BDL, bile-duct ligated; FWHM, full width at half maximum; tCr, total creatine; Glu, glutamate; Gln, glutamine; GSH, glutathione; NAA, N-acetyl-aspartate; CRLB, Cramer–Rao lower bound.*

using two different acquisition techniques. The first one, SPECIAL (15), was used with an ultra-short echo time, while the second one, MEGA-SPECIAL (16), a *J*-difference editing pulse sequence optimized for the first time in this study for Gln detection, was used with a relatively long echo time. Such comparison may in particular help in assessing the impact of a potential short $T_2$ component of the Gln signal on its quantification.

## METHODS

### Protocol

All experiments were carried out on an animal 26 cm diameter horizontal-bore 14.1 T magnet (Magnex Scientific, Oxford, UK) interfaced to a Direct-Drive console (Varian, Palo Alto, CA, USA). The magnet was equipped with a 12 cm diameter gradient (400 mT/m in 120 μs; Magnex Scientific, Oxford, UK). A custom-made quadrature surface coil, consisting of two geometrically decoupled (crossing at a 90° angle) 14 mm diameter single loops, was used as a transceiver.

All animal experiments were conducted according to federal and local ethical guidelines and the protocols were approved by the local regulatory body. Measurements were made on six healthy female adult Wistar rats and six bile-duct-ligated (BDL) female adult rats, an animal model of chronic hepatic encephalopathy (17) characterized by high brain Gln levels at seven weeks after operation (1,18). These rats were used to investigate whether similar results are obtained with different brain Gln concentrations.

Rats were anesthetized with 4% isoflurane combined with a mixture of air and $O_2$, stereotaxically fixed with two ear pieces and a bite bar in a holder and placed at the isocenter of the magnet. Throughout the experiment, the rats were kept anesthetized with 1.5–2% isoflurane. Breathing and temperature parameters were monitored with an MR-compatible monitor system (model 1025; SA Instruments, Stony Brook, NY, USA), and animal temperature was maintained at 37.5–38.5 °C by circulating warm water.

$T_2$-weighted multi-slice images were acquired using a fast spin echo pulse sequence (field of view $30 \times 30\ mm^2$, data matrix $256^2$, slice thickness 0.8 mm, effective TE =52 ms and repetition time TR = 5000 ms) to image brain anatomy in order to locate the volume of interest (VOI).

Before the MRS, the static field homogeneity was adjusted using first- and second-order shims with an echo-planar imaging version of FASTMAP (19).

To obtain a reliable local calibration of the pulse power for the frequency selective editing pulse in MEGA-SPECIAL when the surface coil was used, a novel power calibration was performed. Here, the calibration pulse sequence consisted of a STEAM sequence, in which an editing pulse (10 ms, Gaussian shape) was inserted in the middle of the time period between the second and third STEAM 90° RF pulse. Pulse power adjustments were performed on the water signal. The amplitude of the editing pulse was arrayed in order to obtain a complete nulling of the water signal when the formation of the stimulated echo was suppressed by a perfect 90° editing pulse. To obtain the amplitude of a 180° editing pulse, the amplitude of the 90° pulse was doubled.

Following these adjustments, *J*-difference edited spectra and short echo time spectra were acquired in the same VOI in the hippocampus of each animal ($VOI = 2.2 \times 3 \times 2.5\ mm^3$). The hippocampus was chosen as the region of interest because it is affected in many cerebral disorders. For example, it was shown to be associated with cognitive impairments in an animal model of chronic hepatic encephalopathy (20).

### MEGA-SPECIAL *J*-difference editing spectroscopy

*J*-difference editing was obtained for the $^1$H resonances bound to the C4-glutamate (Glu) and C4-Gln centered at respectively 2.34 ppm and 2.44 ppm via their coupling with the C3-proton resonances of both metabolites centered at respectively 2.08 ppm and 2.11 ppm using the homonuclear *J*-difference editing technique MEGA-SPECIAL (16). MEGA-SPECIAL was implemented by adding an edit-ON/edit-OFF MEGA editing scheme (21) to the SPECIAL pulse sequence (15) as illustrated in Figure 1. During the edit-ON acquisition, single-banded refocusing pulses (10 ms Gaussian pulse with a full width at half maximum, FWHM = 150 Hz) were added to SPECIAL and applied at 2.1 ppm (at the C3-proton resonance position) to selectively refocus the C4-Glu and Gln $^1$H resonances (at respectively 2.34 and 2.44 ppm), while during the edit-OFF acquisition no editing pulse was applied.

#### *Simulations*

In general, an optimal echo time can be chosen for weakly coupled spin systems in order to achieve an inversion of specific lines of the multiplets of interest during the edit-OFF acquisition. The subtraction of both edit-ON and edit-OFF acquisitions therefore allows the detection of only the resonances coupled to the selectively inverted spins, while all other non-coupled resonances, which undergo a similar evolution during both edit-ON and edit-OFF acquisitions, are eliminated. However, in the case of the Glu and Gln spin systems, due to the non-equivalence of the C4 protons and their strong coupling, the determination of the optimal echo time was obtained by numerical simulations using the density

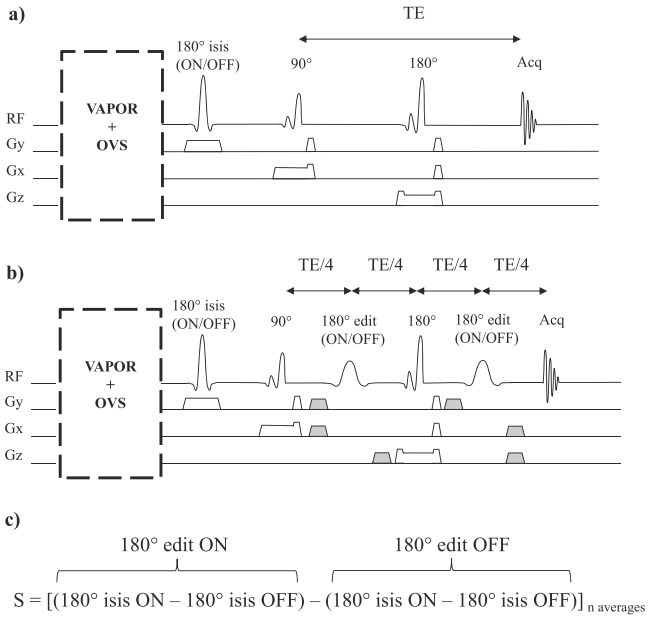


**Figure 1**. (a) Scheme of the SPECIAL pulse sequence. (b) Scheme of the MEGA-SPECIAL pulse sequence where two frequency-selective pulses have been added to the original SPECIAL sequence. The inversion (isis) and editing pulses, which are applied in an alternative ON/OFF scheme during repetitive acquisitions, are indicated by the label ON/OFF. (c) Diagram of one acquisition cycle of the MEGA-SPECIAL sequence, which consists of two interleaved acquisitions of the ISIS scheme that were directly subtracted from each other, while the edit-ON and edit-OFF acquisitions were separately stored before being subtracted.

matrix formalism and the chemical shifts and *J*-coupling constants of the Gln and Glu spin systems reported by Govindaraju *et al.* (22). In the simulation, perfect signal localization was considered, while excitation, refocusing and selective inversion pulses were all simulated as instantaneous rotations with ideal flip angles. MEGA gradients were also omitted from the simulations. Simulations of the edit-ON and edit-OFF spectral outputs were individually performed before being subtracted. The pulse sequence during the edit-OFF scan was modeled as a simple spin echo, while two frequency selective pulses that invert only the C3-proton resonances of Glu and Gln were added before and after the non-selective refocusing pulse to simulate ideal editing during the edit-ON acquisitions. Spin evolution between pulses was described with free precession Hamiltonians that take the chemical shift and *J*-coupling effects into account. The echo time was varied between 27 ms and 127 ms, while the inter-pulse delay was TE/4 (for more details see Fig. 1). The TE of 27 ms was the shortest achievable echo time. To take $T_2$ relaxation into account in the simulations, the simulated signal was then weighted by an exponential function of the form $e^{-TE/T_2}$, where $T_2$ values of Gln (116 ms) and Glu (89 ms) measured at 9.4 T were taken from the work of Xin *et al.* (23). It was shown that $T_2$ values of metabolites are shorter at 14.1 T than at 9.4 T (24). Since this effect is moderate ($T_2$ of Glu is 72 ms at 14.1 T (24)), we assume that the difference in transverse relaxation between Glu and Gln at 14.1 T is similar to that at 9.4 T.

### *Experiment*

To meet the conditions for an optimal echo time and efficiently invert signals of protons between 2.03 and 2.13 ppm (corresponding to a 60 Hz range) coupled to the C4-proton resonances of Glu and Gln, the editing 10 ms Gaussian pulse with an FWHM of 150 Hz was preferred over longer ones with smaller bandwidth. Since the C4 protons of Glu resonate only at a frequency 144 Hz higher than the frequency of the editing pulse, their signal was partially (about 5%) inverted by the editing pulse. To check whether the editing efficiency of the Glu resonance was significantly reduced by this effect, spectral editing was also performed using 15 and 20 ms editing pulses for comparison (an echo time of 45 ms was used for these measurements). The C4 protons of Gln, of which the resonance is located 200 Hz downfield from the center of the editing pulse, were affected by less than 2% of the editing RF pulse amplitude, which was therefore neglected. To optimize the editing efficiency, an array of acquisitions with frequency offsets of the editing pulse in range of 60 Hz around 2.1 ppm was performed to determine the optimal frequency offset. Finally, to avoid any excessive variability of the edited peak amplitudes, the main magnetic field frequency drift was monitored and did not exceed 12 Hz during acquisition.

Edited spectra were obtained after subtraction of the edit-OFF from the edit-ON acquisitions using the acquisition parameters summarized in Table 1. The two interleaved acquisitions of the SPECIAL acquisition scheme were directly added to each other, while the edit-ON and edit-OFF acquisitions were separately stored and frequency-drift corrected before being subtracted.

### *Quantification*

The Gln concentration was quantified relative to the total creatine (tCr = creatine + phosphocreatine) concentration peak at 3.03 ppm in the unedited (edit-OFF) MEGA-SPECIAL spectrum using LCModel (25) and a measured basis set of *in vitro* metabolite spectra acquired with MEGA-SPECIAL using the same parameters as those used for *in vivo* editing measurements. Basis spectra were acquired in pure metabolite solutions in pH buffer (pH = 7, $T$ = 37 °C) with a concentration of 10 mM. In addition to Glu, Gln and tCr, glutathione (GSH) was also included in the basis set, since its resonance located at 2.15 ppm falls into the inversion band of the editing pulse, resulting in the editing of coupled resonances that overlap with the co-edited C2 resonances of Glu and Gln located at 3.75 ppm. Spectral quantification was restricted from 2.1 ppm to 4.5 ppm, which corresponds to the edited spectral region. Since co-editing of the C4-proton resonance of Glu was obtained simultaneously with the Gln resonance editing, the Glu peak may also be used as a reference for Gln quantification, provided that it is efficiently edited. To confirm the validity of this relative quantification, the Gln concentration was therefore also quantified relative to the measured co-edited Glu concentration. No macromolecule edited signal was taken into account in the quantification, since no macromolecule resonance that falls into the editing pulse bandwidth (between 2 and 2.2 ppm) was coupled to macromolecule resonances of sufficient intensity to contribute to the spectrum in the investigated spectral region (26). To confirm that edited macromolecule signals did not contaminate the edited Glu and Gln signals, a metabolite-nulled spectrum was measured *in vivo* after adding an adiabatic inversion pulse before the MEGA-SPECIAL sequence. The inversion time (TI) between the inversion pulse and the beginning of the MEGA-SPECIAL pulse sequence was set to TI = 775 ms in order to minimize Glu and Gln contributions. To further reduce metabolite contributions, which have longer $T_1$ values than macromolecules, the repetition time was also reduced to 2500 ms, such that the magnetization available for excitation after repetitive acquisitions was smaller for the metabolite than for macromolecules.

Relative intensities of the basis-set spectra were calibrated using a phantom containing all the basis-set metabolites with concentration 1.5 mM (GSH), 8 mM (creatine) and 10 mM (Glu and Gln) and using the Gln spectrum as a reference for calibration in a procedure similar to that previously described (27,28). The calibration factor $k_{metab}$ by which each basis spectrum was multiplied was determined as follows:

$$k_{metab} = \frac{c_{gln}\, S_{metab}}{c_{metab}\, S_{gln}},$$

where $S_{metab}$ and $S_{gln}$ correspond to the LCModel quantification values obtained in the calibration phantom using the non-corrected basis-set spectra, and $c_{metab}$ and $c_{gln}$ correspond to the real concentration of the metabolites in the calibration phantom.

The validity of this calibration was confirmed in series of buffered solutions with varying Gln concentrations (1 mM, 2.5 mM, 5 mM) as well as 10 mM Glu, 8 mM creatine and 1.5 mM GSH.

**Table 1.** Summary of the acquisition parameters used for SPECIAL and MEGA-SPECIAL acquisitions

| | SPECIAL | MEGA-SPECIAL |
|---|---|---|
| Echo time [ms] | 2.8 | 27 |
| Repetition time [ms] | 4000 | 4000 |
| Number of transients | 128 | 1024 |
| Acquisition time [min] | 9 | 68 |

*In vivo* validity of the calibration supposes that the difference in $T_2$ relaxation between *in vitro* and *in vivo* measurements is similar for Glu (respectively creatine) and Gln.

### SPECIAL short echo time spectroscopy

*In vivo* short echo time spectra were acquired with SPECIAL (15) as described previously (29). Acquisition parameters are summarized in Table 1. In each animal, the spectral acquisition using SPECIAL was performed right after the one performed with MEGA-SPECIAL and in the same VOI.

Absolute quantification of the spectra was performed using the water signal as an internal reference. The water spectra were acquired with the same parameters, without water suppression and with $n_t = 8$.

*In vivo* $^1$H spectra were processed as previously described (30). Briefly, they were frequency-drift corrected, summed and eddy-current compensated using the water signal from the same VOI.

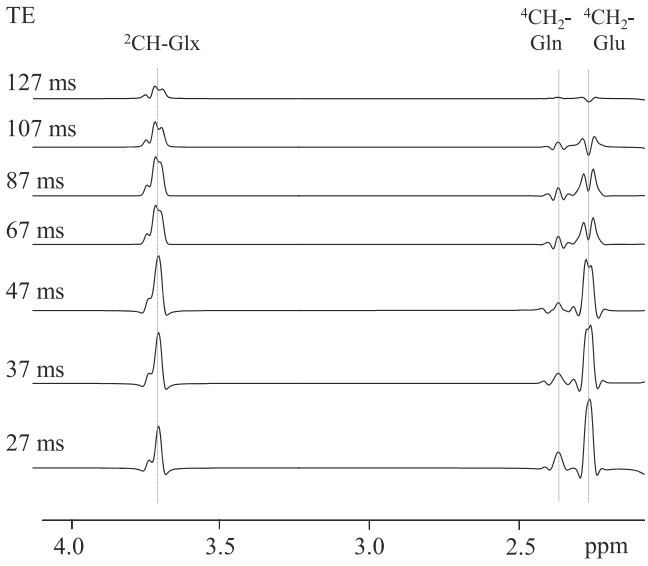


**Figure 2.** Simulations of the difference spectra of the Gln and Glu signals obtained at different echo times with the *J*-difference editing pulse sequence MEGA-SPECIAL by subtracting simulated edit-ON and edit-OFF spectra. The maximal amplitude of the Gln (and Glu) signal was obtained at a TE of 27 ms.

The experimental spectra were fitted with LCModel using a simulated basis set of metabolites and a macromolecule spectrum measured *in vivo* using an inversion recovery sequence as described previously (29). The metabolite basis set included alanine, ascorbate, aspartate, creatine, myo-inositol, gamma-aminobutyric acid, glucose, Gln, Glu, glycine, glycerophosphocholine, GSH, lactate, N-acetyl-aspartate (NAA), N-acetyl-aspartyl-glutamate, phosphocholine, phosphocreatine, phosphorylethanolamine, *scyllo*-inositol and taurine.

### Statistical analysis

To statistically assess the potential significant differences between the quantification results obtained using either SPECIAL or MEGA-SPECIAL, a paired Student *t*-test, which paired the quantification results obtained in the same VOI, was performed between the quantification results obtained with the two acquisition methods.

## RESULTS

Based on numerical simulations, the best echo time for editing the C4-proton Gln resonance was determined to be 27 ms (Fig. 2), which was achieved by decreasing the editing RF pulse length to 10 ms.

To assess the frequency selectivity of the 10 ms editing pulse and a potential signal loss of the C4-proton resonance of Glu located near the editing pulse, a comparison of the editing efficiency obtained using editing pulses of 10, 15 and 20 ms was performed. No observable signal loss of the edited Glu (or Gln) resonance was detected when using the 10 ms pulse instead of 15 and 20 ms pulses (data not shown), implying that a sufficient frequency selectivity was obtained to edit the C4-proton resonances of Gln and Glu simultaneously.

The efficient spectral editing of the Glu and Gln resonances was furthermore confirmed by their consistent detection in edited spectra (Fig. 3). The GSH C4-proton resonance was also consistently detected. The high spectral dispersion obtained at 14.1 T furthermore allowed the clear separation of the three resonances. A residual NAA signal at 2.01 ppm was observed in the edited spectrum (not shown), attributed to the partial saturation of the NAA resonance during the edit-ON scan. However, since the NAA residual was judged to be sufficiently distant from the C4-proton resonances of Glu and Gln, confirmed by a flat fit residual in the frequency range of interest, the NAA signal was not taken into account.

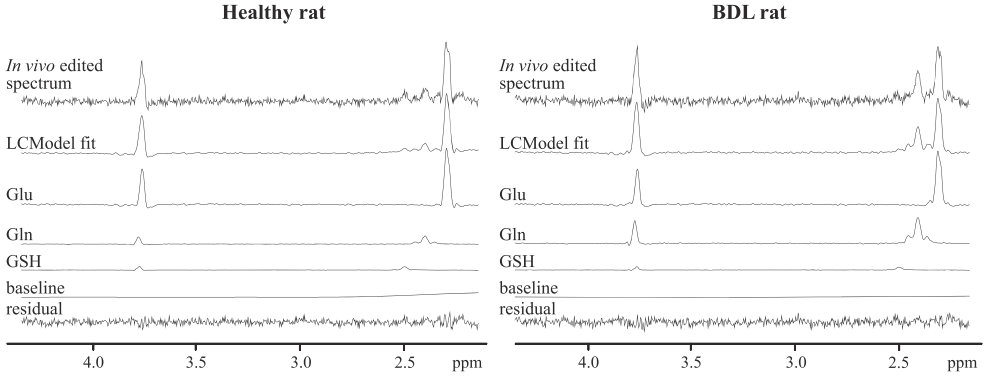


**Figure 3.** From top to bottom: *in vivo* edited $^1$H spectra obtained with MEGA-SPECIAL (TE = 27 ms) in a healthy rat brain (left) and a BDL rat brain (right); the edited spectra are displayed on top of their LCModel analysis, including the fits of the edited metabolites Glu, Gln and GSH. In addition, the baseline and residual of the LCModel fits are also displayed.

All relevant edited resonances that contribute to the spectrum were correctly evaluated in the LCModel quantification process (Fig. 3), as indicated by the fit residual that showed only noise without evidence of unassigned resonances.

Furthermore, the metabolite-nulled spectrum measured *in vivo* did not exhibit any macromolecule resonances in the region of the edited Glu and Gln resonances (Fig. 4), which is consistent with the absence of edited macromolecule signals from this spectral region.

LCModel quantification consistently resulted in quantification of Glu and Gln with Cramer–Rao lower bounds (CRLBs) lower than 5% and 10%, respectively. GSH was also quantified, but with lower precision (CRLB $< 30\%$). LCModel quantification of the 3.03 ppm tCr peak in the unedited spectra was performed with high precision (CRLB $< 7\%$).

To compare the metabolite concentrations measured with SPECIAL and MEGA-SPECIAL in the same VOI, quantification results obtained in the SPECIAL spectra were also evaluated using metabolite concentration ratios between Gln and tCr or Glu, as performed for the edited spectra obtained with MEGA-SPECIAL. The high spectral dispersion obtained at 14.1 T enabled consistent quantification in the spectra acquired with SPECIAL of all the metabolites included in the basis set including the metabolite of interest, namely Gln, Glu and tCr (Fig. 5), which were quantified with CRLB lower than 5%, indicating excellent quantification accuracy.

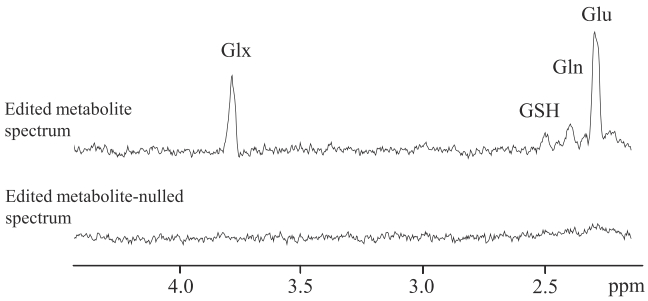


**Figure 4**. Top: an *in vivo* edited $^1$H spectrum acquired with MEGA-SPECIAL at TE = 27 ms is displayed with the edited resonances assigned. Bottom: corresponding metabolite-nulled edited spectrum obtained after the addition of an inversion pulse (TI = 775 ms) that allows the nulling of the edited metabolite signals. No resonances were detected in the metabolite-nulled spectrum.

The Gln to Glu concentration ratios measured with MEGA-SPECIAL ($C_{gln}/C_{glu} = 0.28 \pm 0.04$) and SPECIAL ($C_{gln}/C_{glu} = 0.27 \pm 0.02$) in healthy rat brains were found to be in very good agreement (Table 2). The standard deviation of the average Gln concentration was, however, slightly higher when measured with MEGA-SPECIAL, which reflected the lower quantification accuracy consistent with the higher CRLB, which was ascribed to the lower signal to noise ratio per unit time of *J*-difference editing techniques.

Similar agreement on relative Gln concentrations measured with MEGA-SPECIAL and SPECIAL was found for the BDL rats, which expressed a higher Gln to Glu concentration ratio. This higher Gln to Glu concentration ratio was due to an increase of the Gln concentration in the BDL rats, as indicated by the absolute Gln and Glu concentrations measured with SPECIAL (Table 2). The higher standard deviation of the average Gln concentration in the BDL rat group reflects the intra-group variability due to Gln increases that varied between animals due to varying disease progression.

The results obtained when using the tCr concentration as a reference for Gln quantification were also in very good agreement when measured with both MEGA-SPECIAL and SPECIAL acquisition methods in the healthy and BDL rats.

A quantitative evaluation of the difference in the concentrations obtained from spectra acquired with SPECIAL and MEGA-SPECIAL gave a difference on the order of 4% in the mean values of Gln concentration relative to Glu (and 6% for tCr), which was associated with a standard deviation of 14–16% for both quantification

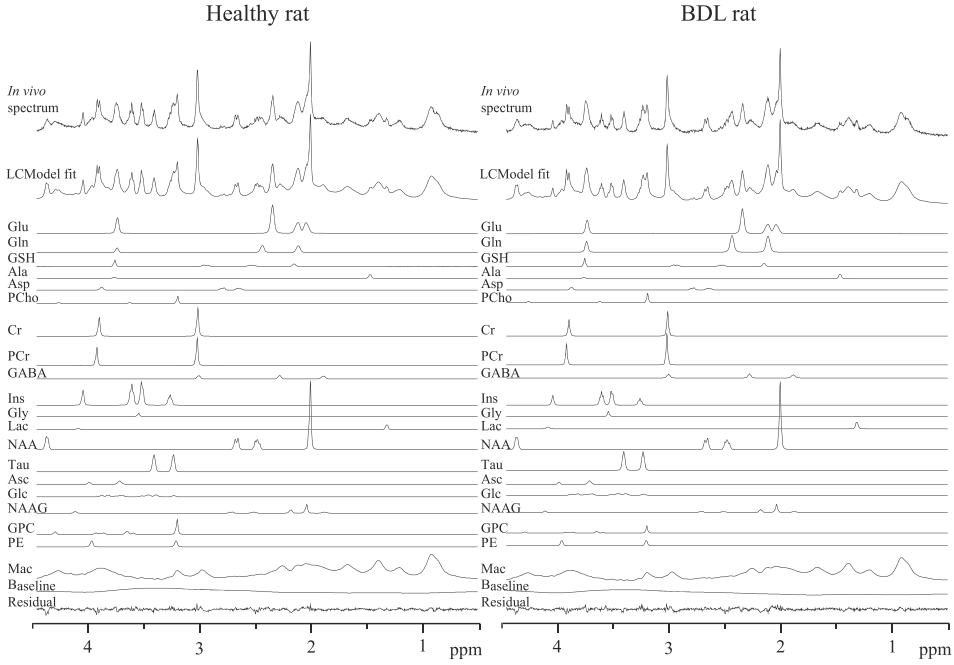


**Figure 5**. *In vivo* $^1$H spectra obtained with SPECIAL (TE = 2.8 ms) in a healthy rat brain (left) and a BDL rat brain (right); the spectra are displayed on top of their LCModel analysis.

**Table 2.** Results of LCModel quantification performed on the *in vivo* rat brain $^{1}$H spectra acquired with SPECIAL at an echo time of 2.8 ms and MEGA-SPECIAL at an echo time of 27 ms.The upper part of the table presents the relative Gln concentrations obtained with both methods, while in the bottom part the results of the quantitative comparison between the quantification results obtained with the two acquisition methods are summarized

| | Healthy rat ($n = 6$) | BDL rat model ($n = 6$) |
|---|---|---|
| *LCModel quantification results* | | |
| $C_{Gln}/C_{Glu\ MEGA\text{-}SPECIAL}$ | 0.28 ± 0.04 | 0.72 ± 0.22 |
| $C_{Gln}/C_{tCr\ MEGA\text{-}SPECIAL}$ | 0.34 ± 0.07 | 0.65 ± 0.20 |
| $C_{Gln}/C_{Glu\ SPECIAL}$ | 0.27 ± 0.02 | 0.70 ± 0.19 |
| $C_{Gln}/C_{tCr\ SPECIAL}$ | 0.36 ± 0.03 | 0.70 ± 0.21 |
| $C_{Gln\ SPECIAL}$ [μmol/g] | 2.75 ± 0.18 | 6.22 ± 1.42 |
| $C_{Glu\ SPECIAL}$ [μmol/g] | 10.15 ± 0.27 | 9.09 ± 0.69 |
| $C_{tCr\ SPECIAL}$ [μmol/g] | 7.68 ± 0.21 | 7.09 ± 0.24 |
| *Relative quantification differences* | | |
| $\frac{C_{Gln}/C_{Glu\,MEGA-SPECIAL} - C_{Gln}/C_{Glu\,SPECIAL}}{C_{Gln}/C_{Glu\,SPECIAL}}$ | 0.05 ± 0.16 | 0.04 ± 0.16 |
| $\frac{C_{Gln}/C_{tCr\,MEGA-SPECIAL} - C_{Gln}/C_{tCr\,SPECIAL}}{C_{Gln}/C_{tCr\,SPECIAL}}$ | - 0.07 ± 0.14 | - 0.06 ± 0.16 |

approaches and using both acquisition methods (Table 2). A paired Student t-test on the Gln concentrations measured in the spectra obtained with SPECIAL and MEGA-SPECIAL indicated non-significant differences between the two measurements ($p = 0.5$ and $p = 0.15$ when using Glu or tCr respectively, as internal reference concentration).

## DISCUSSION

This study aimed to investigate the impact of acquisition parameters on the measured Gln concentration using SPECIAL at ultra-short echo time and MEGA-SPECIAL at a moderate echo time. The comparison of the Gln concentration measurement obtained using both methods allowed in particular the assessment of the potential impact of a short $T_2$ component of the Gln signal on its quantification in the investigated echo time range.

To this end, this study focused on the achievement of an optimal detection of the Gln signal. First of all, the measurement of the Gln signal was optimized in MEGA-SPECIAL by using an optimal echo time for the Gln signal detection, as well as by carefully calibrating the editing pulse frequency offset and power to maximize editing efficiency. While efficient Glu co-editing was also shown to be feasible when using optimized parameters, keeping frequency drift within the ~12 Hz range should furthermore have limited loss of editing efficiency.

Second, the use of an ultra-high magnetic field strength of 14.1 T provided an increased spectral dispersion that allowed improved individual detection of the C4-proton resonances of the Glu and Gln signals and their reliable individual quantification. SPECIAL and MEGA-SPECIAL acquisition methods provided similar relative Gln concentrations, which were quantified with low CRLB (<10%) when using either method (Table 2). The Gln concentrations obtained at ultra-short echo time using SPECIAL were further in excellent agreement with previous studies performed in the healthy rat brain at high field with similar echo times (31–34).

Moreover, it has been observed in previous studies that an incorrect estimation of macromolecule contribution can significantly affect the measurement of the metabolite concentration (31,35), indicating that their accurate assessment was necessary. The macromolecule contribution to the $^{1}$H spectra was therefore incorporated in the quantification as a measured basis-set signal in the case of the SPECIAL acquisitions. In the case of the MEGA-SPECIAL acquisitions, the possible contamination of the edited metabolite signals with co-edited macromolecule signals was assessed by acquiring an edited macromolecule spectrum. The results obtained showed the absence of edited macromolecule signals in the frequency range of interest (Fig. 4), which was in agreement with the absence of reported *J*-coupling between macromolecule resonances in the edited region and in the excitation range of the editing pulse (26). This result confirmed that no macromolecule contribution had to be taken into account in the quantification of the edited spectra acquired with MEGA-SPECIAL.

To eliminate the presence of a quantification bias induced by an inaccurate editing of the C4-proton Glu resonance due to its position close to the inversion band of the editing pulse, the Gln concentration was also measured relative to the tCr concentration. The highly consistent results obtained when using either metabolite as a reference suggest efficient and stable simultaneous editing of Glu along with Gln. This was further supported by the standard deviation of the Gln concentration measurement being similar when using tCr or Glu as a reference. These results confirm the validity of Gln quantification using co-edited Glu as a reference, and in turn the reliable quantification of the co-edited Glu signal.

Very good agreement was obtained between the Gln measurements performed with SPECIAL at ultra-short echo time (TE = 2.8 ms) and MEGA-SPECIAL at longer echo time (TE = 27 ms), as indicated by the small and statistically non-significant quantification differences obtained between the Gln concentrations measured by the two methods, as shown in Table 2. Indeed, less than 5% difference of the Gln concentration was obtained between the two measurements when Gln was quantified relative to Glu, and 7% when quantified relative to tCr. Furthermore, the mean difference of the measured Gln concentration using the two methods was of the order of the standard deviation of the Gln concentration measured with SPECIAL.

This agrees well with the results of statistical tests, which showed that no measurement bias between the two methods could be detected within experimental error. Moreover, the difference of the Gln concentrations measured with the two

acquisition methods at different echo times was similar for both the healthy rats and the diseased rats, which were characterized by higher brain Gln concentrations. This suggests that the lower Gln concentration measured in the healthy brain was not systematically biased. Furthermore, the very good agreement obtained in this study between the two measurements of the Gln concentration made at short and longer echo times indicates that there is no evidence of an underestimation of the Gln concentration at moderate echo time due to an effect of an additional short $T_2$ component of the Gln transverse relaxation in *in vivo* conditions due to several brain compartments, as hypothesized by Hancu and Port (7). The results of this study are further supported by results from previous studies that reported measured Gln concentrations in the human brain in the higher range when using long echo times (TE $>$ 30 ms) (14). In addition, it is of interest that the Gln concentration that we report here for the rat brain is close to the $2.2 \pm 0.4$ μmol/g reported by Mekle *et al*. (13) for the human brain at 7 T using the SPECIAL technique with a short echo time.

Since similar Gln concentration results were obtained when using either SPECIAL or MEGA-SPECIAL, it also indicates that SPECIAL appears to be as reliable as a *J*-difference editing pulse sequence for measuring the Gln concentration and might therefore be considered as a reliable method for Gln measurement, while having several advantages over MEGA-SPECIAL. The SPECIAL spectroscopy, which has already been successfully implemented at 3 T and 7 T (13), provides concentrations of all the metabolites in the neurochemical profile. In addition, it gives an inherently higher SNR per unit time compared with MEGA-SPECIAL, which suffers from longer echo times and RF pulse imperfections.

We therefore conclude that the impact on the measured Gln concentration of using the different acquisition techniques SPECIAL and MEGA-SPECIAL at echo times less than 30 ms is negligible and that there is no evidence of a systematic underestimation of the Gln concentration in the investigated echo-time range due to a short $T_2$ component in the transverse relaxation of the Gln signal.

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