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H-Spectroscopy Measurements of Glutamine and Glutamate

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Abstract— Introduction: Glutamine (Gln) and glutamate (Glu) signals are highly overlapped in the region of 2-2.5 ppm in human brain due to the resemblance of their spin systems, making it difficult to separate these metabolites using short-TE PRESS or STEAM at 1.5 T. These metabolites are highly important markers for many diseases, and accurately estimation of them is important. **Methods**: This study reports the detection of Glu and Gln at 3 T, and immune to the frequency drift. The echo times were optimized, with numerical analysis of the filtering performance, as {TE(1), TE(2), TE(3)} = {23, 74, 18} ms. The amplitude ratios of the filtered Glu and Gln multiplets with respect to 90°-acquisition were 28% and 60%, respectively. **Results**: Calculation indicated that the amplitude of filtered Gln peak is two-fold compared to STEAM scheme, regardless of the relaxation effects. Glu and Gln are detected at 2.29 ppm and 2.39 ppm simultaneously, without substantial interferences from the neighboring resonances *in vivo*. The filtered sum spectra with and without Gln in LCModel analyses, using density-matrix calculated spectra as basic functions. The concentration of Glu and Gln in hippocampus of healthy adults was estimated to be 2.8±0.7, and 10.5 ±0.6 mM respectively, and NAA of 9.7±0.6 mM, with reference to creatine at 8 mM. The fit standard deviations are10± 2%, 6±2 % and 2±1% respectively. **Conclusion**: The presented result shows the fully resolved and Glu and Gln which helps in accurate estimation of them in different diseases.

Keywords: Glutamine, Glutamate, Neurotransmitters, Spectroscopy, PRESS, STEAM.

Introduction

Glutamate (Glu) and glutamine (Gln) are two primary neurotransmitters in the central nervous system. Glutamate, the most abundant neurotransmitter in the brain, plays a central role in nitrogen metabolism and participates in multiple biochemical pathways. Glutamine (Gln) is mostly located in glial cells as the end product of Glu catabolism and is a reservoir for Glu production in the neuron. These two free amino acids involved in neuronal function, plasticity, metabolism, and excito-toxicity [1, 3, 4-7]. Overload Glu in the synaptic space induces neuro-toxicity, which has been linked to many degenerative diseases, including amyotrophic lateral sclerosis (ALS) [8], Alzheimer's disease (AD) [9, 10], multiple sclerosis (MS) [11], Huntington's disease (HD) [12], and to neuronal apoptosis injury, such as traumatic brain injury (TBI) [13],and stroke [14]. Since glutamate-glutamine cycle plays a role in the neuronglia communication in the synapse, it seems that impairment of glutamate-glutamine cycle implicated in the pathophysiology of schizophrenia, where many evidences suggested that a dysfunction in glutamatergic neurotransmission might be involved in the patho-physiology of schizophrenia. Many MRS studies of schizophrenic patients reported a significant increase in glutamine level in the medial prefrontal cortex of never-treated

schizophrenic patients compared with controls, while others reported reduction of cerebrospinal fluid (CSF) levels of glutamate in patients with schizophrenia [15-21]. Gregor Hasler in 2007 [22], suggested that major depressive disorder (MDD) is associated with perturbations in the metabolism of the major excitatory and inhibitory neurotransmitters glutamate and gamma-aminobutyric acid (GABA), respectively. More recent neuro chemical studies concluded that severe depression is accompanied by perturbation of the metabolism of excitatory amino acids, especially of glutamate (Glu) [23-25]. Auer et al. in 2000 [21], reported a significant reduction of glutamate-glutamine (Glx) in the bilateral anterior cingulum in severely depressed patients. Colla et al [26], 2008 reported an increase of Hippocampal glutamate concentrations for patients with chronic bipolar disorder. Other studies pointed out that Glu and gln play a major role in patients with psychiatric disorders, such as idiopathic generalized epilepsy [27].

Consequently, we can conclude that noninvasive detection and accurate quantification of glutamate and glutamine have important clinical implications for the treatment of these various diseases. However, resolving and reliable detection of Glu and Gln using proton MR spectroscopy remain challenging at clinically available field strengths (1.5 T), where at 1.5 T (the most clinically field strength accessible), the resonances of these metabolites in the region of 2.2– 2.5 ppm, along with GABA, are completely collapse and mostly assigned to a mixture designated as Glx. Most reliable studies that reported resolved resonances of glutamate and glutamine used field strengths at 3 T or higher. Many methods proposed efficient detection of Glu and/or Gln *in vivo*, mainly at low- and mid-field strengths including spectral editing techniques, such as J-refocused editing and coherence transfer methods [6, 28]. The multiple quantum coherence filtering method [29-31], the Carr-Purcell train refocusing method [32], and the spectrally selective refocusing method, which selectively measures Glu or Gln in separate scans [6]. 2D spectroscopy, such as COSY and 2D J-resolved techniques [33], are among methods which recently developed and used for better resolving of the overlapping metabolites in the 1-4 ppm region [34-36].

Previous MRS method have been utilized to detect, and resolve Glu and Gln. These methods such as the chemical-shift-selective filter method [37], the constant time PRESS (CT-PRESS) [38], optimizing the conventional sequences PRESS sequence with an echo time (TE) of 80 ms for detection of Glu at 3T. Standard STEAM [39] sequence involves two inter-pulse timings TE and the mixing time (TM), with around (TE, TM) (80 ms, 50 ms) for detection of Glu, Gln, and GABA at 4T [1].

This article report the feasibility of using 1H-sepctroscopy at 3T for simultaneous detection of Glu and Gln which proven to be capable of fully-refocused signal return, and immune to the frequency drift.

Method and Materials

Spectral refocusing using PRESS sequence is used to resolve the overlapped C4 resonances of Glu and Gln at 3 T, in the regions of 2.25-2.45 ppm. As shown in Figure 1, only the signal from that target resonance will come out, while other signals suppressed. In this sequence, users can

control echo times (TE1, TE2, TE3), parameters such as shape, duration, flip angle, carrier frequency, and gradient parameters as slope, length and strength.
Sub-echo times optimized, for simultaneous detection of the Gln and Glu C4 proton resonances, with density matrix simulation, which incorporating slice-selective RF and gradient pulses. An echo time set, (TE1, TE2, TE3) of (23, 74, 18) ms, was obtained to reduce the overlap between Gln and NAAAsp, maximize the Gln peak amplitude, and most importantly eliminate the overlap between Gln and Glu by suppressing the Glu signal. Published chemical shifts and coupling constants [3] used. Phantoms and *In vivo* tests of the filtering sequence conducted.

Five phantoms of 3 mM Gln, 10 mM Glu, 1 mM GSH, 10 mM NAA, and 1 mM GABA were prepared, and one phantom contains all these metabolites with the same concentrations. All phantoms solutions have pH of 7.3-7.4. Cr, at 8 mM concentration, was added to all phantoms for frequency references and absolute concentration purposes.

Nine healthy subjects, nine schizophrenia patients on medication (SV-ON), and three schizophrenia patients not in medication (SV-OFF) aged from 25-40 years old, were scanned. Written consent obtained prior to each scan. All phantoms and in vivo tests were carried out using 3 T Philips system, with a quadrature birdcage head coil for RF transmission and reception, a 50×15×15 mm^3 was selected in the hippocampus of each volunteer and 25 x 25 x 25 were selected for phantoms measurements. MP-RAGE (*m*agnetization *p*repared *ra*pid *g*radient *e*cho) images used for in vivo voxel positioning. TR was 2000 ms, spectral bandwidth of 2500 Hz, and 2048 number of sampling points with 4 averages. The water signal was suppressed using WET [40] with four 28 ms long RF pulses (83.6, 98, 74, and 158), and 600 Hz bandwidth. The non-space selective 180° RF pulse with Tp = 60 ms (65 ms for phantom) and BW = 270 Hz, tuned to 2.4 ppm, was applied between the slice-selective 180° radiofrequency (RF) pulses of PRESS to refocus the resonances between 1.3 and 3.5 ppm. Spatial localization was obtained with a 6.6 ms 90° RF pulse (BW = 6.3 kHz) and two 8.9 ms 180° RF pulses (BW = 1.9 kHz). LC Model software [41] used for data analysis.

Data Processing and Spectral Quantification
The final Spectral quantification of the single-voxel 3T data obtained using LCModel, which uses a linear combination of individual metabolite model spectra in spectral analysis. The model spectra obtained either in vitro or from spectral simulation. Metabolites concentrations estimated from their dominant resonance by fitting the range1.0 - 4.1 ppm, and normalized to Cr concentration of 8 mM. The standard deviation for individual measured metabolites of the fitting procedure obtained by Cramér-Rao lower bounds (CRLB) [42]. This routine offered in the LCModel fitting indicates the good agreements between the experimental and fitted data, and returned as percentage standard deviation (%SD).

Results and Discussion
The presented in vivo concentrations of cerebral metabolites were obtained by examinations of brain hippocampus performed in 9 healthy and schizophrenia patients (On and OFF medication) adults aged 25-40 years. The study performed at 3.0 T with use of head coil to ensure homogeneous radio-frequency excitation and signal reception. Proton MRS Spectra

were obtained with three echo times of (18, 23, 74 simulation and in vivo, and 20, 20, 75 in phantom) that ensure to minimize the glutamate glutamine overlapping and maximize the glutamine concentration. Concentrations obtained by means of metabolites fitting procedure using CLModel software. The results provide a quantitative basis for studies of both in vivo normal human and SV patients.

Figure 1 shows the calculated spectra for the studied metabolites using matrix simulation. Well separated glutamate and glutamine from the NAAAsp multiplet at 2.29 and 2.39 ppm. The Glu C4 proton resonance peak is at 2.29 ppm, with negligible overlap with the Gln peak. The amplitude ratios of the filtered Glu and Gln multiplets with respect to 90°-acquisition were 28% and 60%, respectively. A numerical calculation indicates that the amplitude of this filtered Gln peak is two-fold compared to that from the reported STEAM scheme [2], ignoring relaxation effects. It is predicted from the filtered sum spectra with and without Gln in Figure 2 that Glu and Gln can be detected simultaneously without substantial interferences from the neighboring resonances *in vivo*.

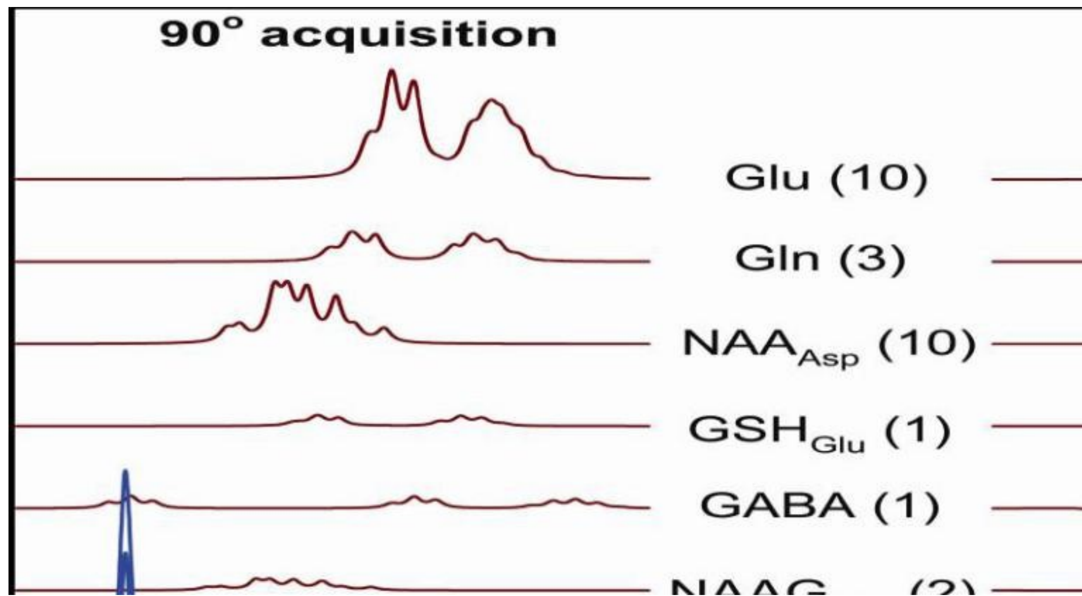
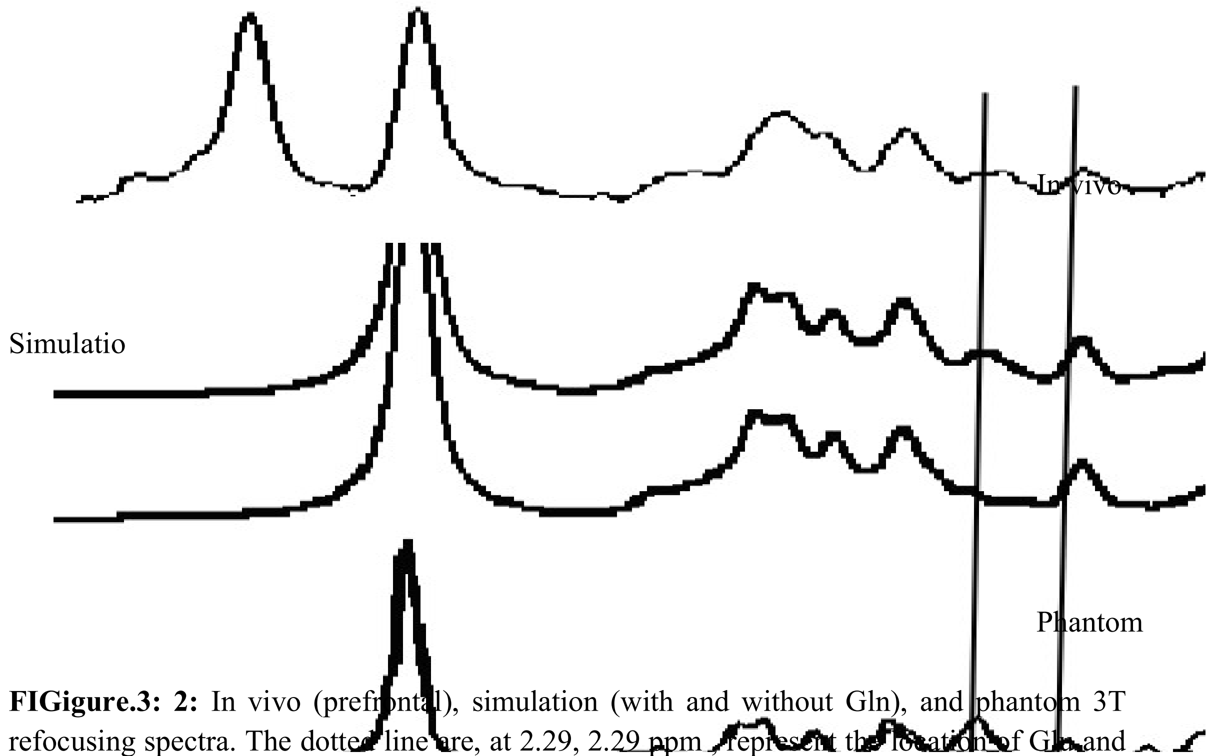


Figure. 1: Calculated spectra of Glu, Gln, NAA, GSH, GABA, NAAG and Cr for a concentration ratio of 10:3:10:1:1:2:8 respectively (using matrix simulation), following 90º-acquisition and triple refocusing. The sub-echo times of triple refocusing TE1, TE2, and TE3 are 23, 74, 18 ms respectively. The presence and absence of Gln make a noticeable difference in triple refocused spectra (indicated by an arrow). The Glu and Gln resonances at 2.29 and 2.39 ppm indicated by vertical lines. Spectra are broadened to 0.04 ppm (5 Hz) for clarification

The LC model fit result of the refocused spectrum shown in Figure.2, and Figure 3 presents filtered *in vivo* brain spectra from five healthy subjects. For the five healthy subjects, the concentrations of Gln, Glu and NAA in hippocampus were estimated to be 2.8±0.7, 10.5±0.6

and 9.7±0.6 mM with reference to Cr at 8 mM, with fit standard deviations of 10±2%, 6±2% and 2±1% respectively.



FIGigure.3: 2: In vivo (prefrontal), simulation (with and without Gln), and phantom 3T refocusing spectra. The dotted line are, at 2.29, 2.29 ppm , represent the location of Glu and Gln peaks respectively. all the spectra were calculated using total TE = 115 ms. Note the good agreement in the metabolites spectra pattern between the simulated and measured data. The similar spectra pattern between the invivo, simulation, and phantom ensures the fecibility of the triple refocusing method of refocusing the spectra of glutamate and glutamine peaks.

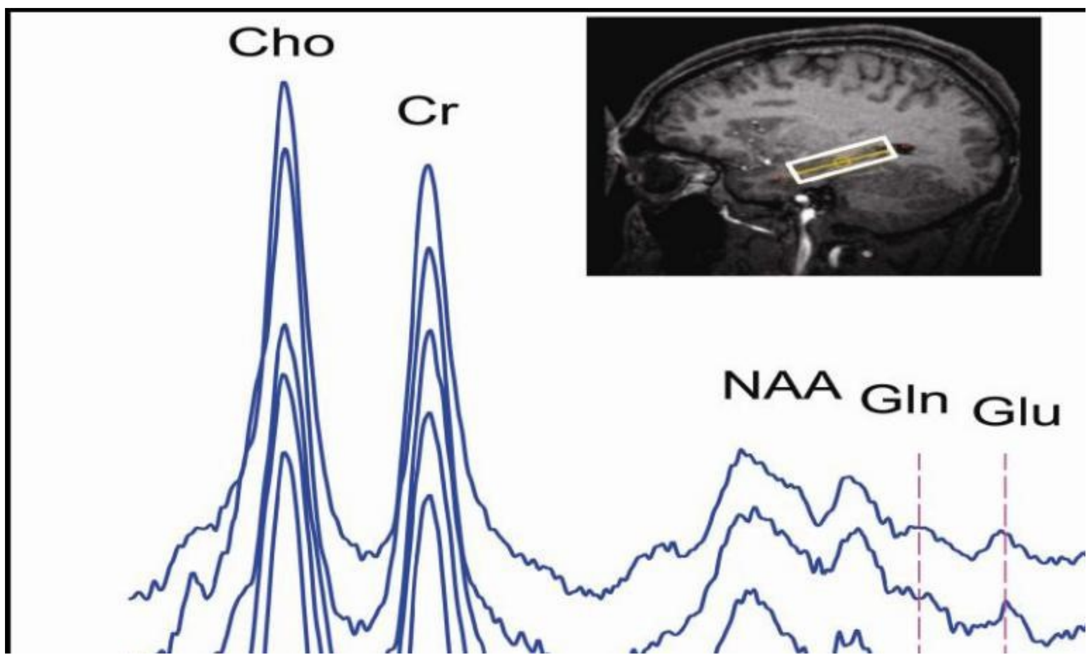


Figure.3: *In vivo* human brain spectra from the hippocampus (50x15x15 mm3) of 5 healthy subjects, obtained with triple refocusing. TR = 2 s. TE = 115 ms. NEX = 512.

Figure 4 represents the LCModel fitting to the 5 in vivo spectra, notice that exact fit which shoe the viability of this method of resolving the glutamate and glutamine peaks, which make the fitting more accurate, that what the clinical needs to use this method in their daily routine.

Figure 4: LCModel analysis of a triple-refocused spectrum. This LCModel fit shows the good agreements between the experimental and the fitting data.

Table 1 presents the calculate concentration of the Gln and Glu along with other metabolites estimated by CLModel fitting method for phantoms and in vivo measurements. A previous [1] estimated concentration values for these metabolites are presented for comparison. As shown in this table the presented concentration of metabolites are similar the previous ones calculated by different method.

Table1: The metabolites concentration as estimated by LCModel. The concentrations referenced to Cr of 8mM. Concentration of the same metabolites obtained by steam optimizations sequence [1] presented for comparison. Note the ability of the triple refocusing method of retain full metabolites signal which is reflect in the final concentration quantification.

Presented measure ment		**Concentration ratio to Cr ± SD**							
	Subject	**Cr**	**Glu**	**Gln**	**Cho**	**Asp**	**NAA**	**GSH**	**NAAG**

	NC	1±2.4	1.6±9	0.52±11	1±3.3	0.3±24	1.0±3.3	0.27±14	0.14±26
	SV-ON	1±3	1.6±8	0.58±12	1.1±3.8	0.33±24	1.1±3.8	0.32±13	0.26±19
	SV-OFF	1±2.6	1.4±9.6	0.42±17	1.2±3.3	0.24±30	1.2±3.3	0.2±27	0.15±26
Ref. [1]	**NC**	1±0.27	1.15±0.69	0.64±0.55	0.17±0.04		1.28±0.69		

Conclusion:

We have demonstrated, through simulation calculations and experimental validation for phantoms and in vivo, that the Glutamate C4 multiplets proton resonance around 2.29 ppm and the Glutamine C4 multiplets proton resonance around 2.39 ppm can be resolved 3 T field strength. The advantages of the proposed approach are full signals resolved of these important amino acids, fully refocused signal return, impervious to frequency drift, and easy to implement since the frequency selective pulse are identical to the two slice selective pulses of normal PRESS sequence. Full resolving of these metabolites will open the door for accurate quantification of their concentration in many neuron diseases, which will be a great help in the clinical trials that based on these concentration in many treatments.

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