

Metabolite Concentration Estimates in the Rat Brain by Magnetic Resonance Spectroscopy Using QUEST and Two Approaches to Invoke Prior Knowledge

C. Cudalbu¹, S. Cavassila¹, H. Ratiney¹, O. Beuf¹, D. van Ormondt², D. Graveron-Demilly¹

¹Laboratoire RMN, CNRS UMR 5012, Université Claude Bernard LYON I-CPE, France.

²Applied Physics Department, Delft University of Technology, Delft, The Netherlands.

Phone: 33+(0)472448208, Fax:33+(0)472448199

Cudalbu.Cristina@univ-lyon1.fr, Sophie.Cavassila@univ-lyon1.fr

Abstract— Localized brain proton spectroscopy can non invasively provide biochemical information from distinct regions of the brain that can be used for disease detection, disease progression monitoring and treatment. Quantitation of Magnetic Resonance Spectroscopy (MRS) signals acquired at short echo time is difficult due to the overlap of metabolite resonances, macromolecules and lipids and to the presence of the water residue. Moreover, the signals have low signal-to-noise ratio. A fitting algorithm invoking extensive prior knowledge is then needed. Metabolites were quantitated using the method QUEST which fits a combination of metabolites signals from a basis set to the *in vivo* data. The basis set can be obtained either by quantum mechanically simulating the theoretical metabolite signals, or by measuring signals of metabolite aqueous solutions. In this paper, we compare the influence of the basis set on the quantitation results. Short echo-time *in vivo* signals of rat brains were acquired at 7 Tesla from the central region of the brain containing the striatum and thalamus. Estimated brain metabolite concentrations are compared for the two basis sets and to previously reported results.

Keywords— Magnetic Resonance Spectroscopy (MRS), Signal Processing, Rat Brain Metabolites, Quantitation.

I. INTRODUCTION

Localized brain proton Magnetic Resonance Spectroscopy (MRS) can non invasively provide - by quantitation of brain metabolites - biochemical information from distinct regions of the brain that can be used for disease detection, disease progression monitoring and treatment. Using short echo-time localization pulse-sequences to investigate rat brains, the observation of metabolites with short spin-spin relaxation decay time constants and with coupled spin systems is

possible. Reliable metabolite quantitation is difficult and faces various challenges. Three major problems are encountered: (1) strongly overlapping metabolite resonances (many hundreds); (2) low signal-to-noise ratio (SNR) and limited acquisition time; (3) a broad, partially known background component originating mainly from macromolecules and lipids that overlaps the metabolite peaks. Moreover, the (residual) water peak needs to be removed. Fitting of time or frequency-domain model function to such low-SNR *in vivo* data requires extensive prior knowledge.

Two alternative approaches in the time-domain or frequency-domain are used to invoke extensive prior knowledge, (1) measured signals/spectra of selected metabolite aqueous solutions used as numerical time/frequency domain model functions [1, 2, 3]; (2) theoretical metabolite signals/spectra quantum mechanically simulated for the corresponding measurement protocol [1, 2, 4].

The purpose of the present study is to compare the influence of the basis set on the quantitation results. To the best of our knowledge, such a study has not been reported yet. Short echo-time *in vivo* spectra of rat brains were acquired at 7 Tesla from the central region of the brain containing the striatum and thalamus. Metabolites were quantitated with the method QUEST [1] combined with the ‘Subtract’ approach for background modelling, using either an *in vitro* or a theoretical basis set. Brain metabolite concentrations estimated from fifteen rat brain signals are compared to published results. The reliability of our concentration estimates was assessed using the Cramér-Rao lower bounds (CRBs), which reveal the quantitation precision.

II. METHOD

A. Animals

Healthy adult rats (Sprague-Dawley, 320g in weight, 3 month old, 8 animals) were anesthetized by inhalation of isoflurane (Abbott Laboratories, Rungis, France) with 2.5% concentration in a mixture of 50% oxygen and 50% nitrous oxide. The body temperature was maintained at 37°C by warm water circulation. Monitoring of respiratory cycle was performed using an air pillow. Experiments were conducted according to the procedures approved by the institutional animal care and ethical committee of our University.

B. Experimental conditions

All the experiments were performed on a 7T Biospec BRUKER system -ANIMAGE platform, Lyon, France, equipped with a gradient coil (12 cm diameter, 400 mT/m maximum amplitude, 80 μ s minimum rise time). A bird cage coil (72 mm inner diameter) for excitation and a receiver surface coil (15 mm diameter) were used.

All first- and second- order shim terms were adjusted using FASTMAP [5] for each voxel of (3.5mm)³ positioned in the central region of the brain containing the striatum and thalamus. The water signal was suppressed by variable power RF pulses with optimized relaxation delays (VAPOR) [6]. Outer volume suppression combined with a PRESS sequence (bandwidth of 4 kHz, 4096 data points, 128 averages, 5s repetition time, 20ms echo time, acquisition time of 11 min.) was used for localization.

The position of the voxel was based on multislice RARE images (FOV=2.56x1.92 cm, slice thickness 0.5 mm, echo spacing 19.2 ms, echo time 61.2 ms, repetition time 6.2 s). The size of the voxel (3.5mm)³ was adjusted to fit the anatomical structure of the selected brain region and to minimize partial volume effects.

C. Processing Algorithm

Quantitations were performed with QUEST combined with the ‘Subtract’ approach for background modelling.

QUEST fits a *time domain* model function, combination of M (quantum-mechanically simulated or measured) signals of metabolites x^m , $m = 1..M$, directly to the low SNR *in vivo* data. This non-linear least squares algorithm adjusts the model function parameters (a_m , $\Delta\alpha_m$, $\Delta\omega_m$, $\Delta\phi_m$, ϕ_0 , t_0) such that the distance between the raw signal and the estimated signal \hat{x} be minimum:

$$\hat{x}_n = \exp(i\phi_0) \sum_{m=1}^M a_m x_n^m \exp((\Delta\alpha_m + i\Delta\omega_m)t_n + i\Delta\phi_m)$$

where a_m are the amplitudes to be estimated, the amplitudes represent the relative proportions of the M

metabolite signals x_n^m , ($n=0..N-1$ where N is the number of data-points) in the signal x rather than the amplitudes of individual spectral components. The parameters $\Delta\alpha_m$, $\Delta\omega_m$, $\Delta\phi_m$, are small extra damping factors, angular frequencies and phase shifts which enable to automatically compensate for distortions due to the magnetic field heterogeneities. $t_n = nt_s + t_0$ are the sampling times, in which t_0 is the dead-time of the receiver and t_s the sampling interval; ϕ_0 is an overall phase, and $i^2 = -1$.

The numerical time-domain model functions x^m of eleven metabolites [Aspartate (Asp), creatine (Cr), choline (Cho), γ -Aminobutyric acid (GABA), glucose (Glc), glutamate (Glu), glutamine (Gln), N-Acetylaspartate (NAA), taurine (Tau), lactate (Lac) and myo-inositol (Ins)] used as prior knowledge in the algorithm, were obtained using two approaches: (1) an *in vitro* basis-set made of the measured signals of aqueous solutions of the mentioned metabolites and (2) a *simulated* basis set made of the theoretical metabolite signals quantum mechanically simulated.

The reliability of metabolite quantitation was assessed using the Cramér-Rao lower bounds (CRBs). CRBs are widely used in signal processing as a measure of precision for the estimates [7].

It is important to emphasize that QUEST automatically and partially compensates for the spin-spin relaxation effects occurring during the MR sequence duration when an *in vitro* metabolite basis set is used as prior knowledge. The *in vivo* signal weighted by $\exp(-TE/T_{2vivo})$ is modelled by a weighted sum of *in vitro* metabolite signals themselves weighted by $\exp(-TE/T_{2vitro})$, where $T_{2vivo} = 1/\alpha_{vivo}$ and $T_{2vitro} = 1/\alpha_{vitro}$ correspond to the *in vivo* and *in vitro* transverse relaxation times (inverse of the damping factors) of the considered metabolite, respectively and TE is the echo time. The difference between the *in vivo* and *in vitro* T_2 effects can be corrected by multiplying each estimated concentration by $\exp[TE(1/T_{2vivo} - 1/T_{2vitro})]$ if T_{2vivo} and T_{2vitro} are known for each spectral component.

When a simulated basis set is used, QUEST automatically compensates for spin-spin relaxation time effects only if the T_2 effects are taken into account during the simulation of the full measurement protocol.

Since in this study, we compare the influence of the two metabolite basis sets on the quantitation results, we corrected only the simulated basis set signals for *in vitro* T_2 relaxation time effects. It was not necessary to correct for the difference between the *in vivo* and *in vitro* T_2 effects, which require the knowledge of T_{2vivo} .

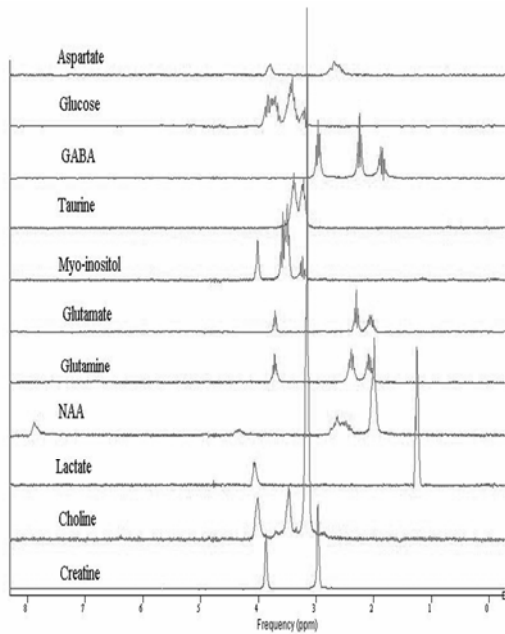


Figure.1a. Metabolite basis set signals acquired *in vitro* at 7 Tesla using a PRESS sequence with an echo time of 20 ms.

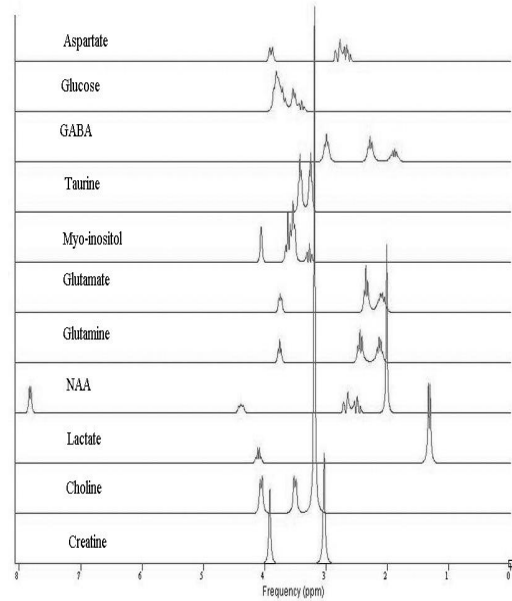


Figure.1b. Metabolite basis set signals quantum-mechanically simulated with NMR-SCOPE using the *in vivo* measurement conditions.

D. *In vitro* metabolite basis set

To set up the *in vitro* metabolite basis set, eleven metabolite solutions were prepared. Asp, Cr, Cho, GABA, Glc, Glu, Gln, NAA, Tau, Lac and Ins (Sigma-Aldrich) were dissolved separately in aqueous solutions (100mM, pH=7.0±0.1, 10ml). Trimethylsilylpropanesulfonic acid sodium salt (DSS) and sodium formate were added as chemical shift references. It is essential that the eleven *in vitro* metabolite signals be measured using identical acquisition parameters as the *in vivo* ones, and the same pH and temperature conditions as the ones in the rat brain. The *in vitro* metabolite basis set used is displayed in Figure 1a.

E. Simulated basis set

Signals of the metabolites were quantum mechanically simulated for the *in vivo* experimental protocol (PRESS sequence, TE=20ms, bandwidth of 4 kHz, 4096 data points) with NMR-SCOPE [8]. The spin Hamiltonian parameters used (number of spins, chemical shifts, J couplings) were obtained from [9]. The simulated metabolite basis set used is displayed in Figure 1b. The present version of NMR-SCOPE does not accommodate T_2 effects during the MR sequence except during the acquisition time.

Since the *in vitro* basis set takes into account the *in vitro* spin-spin relaxation time effects, for comparison, the simulated basis set signals need to be corrected for the spin-spin relaxation time effects too. The latter were corrected using the metabolite *in vitro* relaxation times

$T_{2\text{in vitro}}$ given in [10] by multiplying each signal by $\exp(-TE/T_{2\text{in vitro}})$.

III. RESULTS

The striatum and thalamus region from healthy rat brains was investigated at 7Tesla.

The multislice RARE sequence provided high-quality images of the rat brain that guaranteed precise and reproducible placement of the voxel. In addition to commonly observed NMR signals of the methyl resonances of NAA, Cr and Cho, characteristic spectral patterns of other metabolites, such as: Ins, Tau, Gln, Glu, Asp, Glc, GABA were discernable in ^1H NMR spectra from the studied region. This high spectral resolution was achieved using FASTMAP automated shimming. Shimming resulted in unsuppressed water signal line widths of 8-14 Hz.

Fifteen brain signals from eight rats were processed using the jMRUI software [11, 12]. Removal of residual water components of the *in vivo* and *in vitro* basis set signals was performed in a preprocessing step using the Hankel-Lanczos Singular Value Decomposition algorithm - HLSVD (25 spectral components were used for modelling). The signals were quantitated with 'Subtract'-QUEST using an *in vitro* or a theoretical basis set, respectively. The number of truncated initial data-points which is an important parameter for background

removal in the method ‘Subtract’-QUEST, was equal to 20-25 (corresponding to a duration of 7ms). The zero-order phase and the dead time were fixed to zero.

As previously mentioned, the simulated basis set was corrected for *in vitro* T_2 relaxation time effects by multiplying each signal by $\exp(-TE/T_{2\text{vitro}})$. The $T_{2\text{vitro}}$ values were estimated for the singlets of the three principal metabolites, NAA, Cho, Cr, to be around 400ms [10]. Since, we were not able to estimate the *in vitro* T_2 relaxation times for the other metabolites which have coupled spin systems, we supposed that they had the same $T_{2\text{vitro}}$. For the sake of comparison, metabolite concentration estimates were set proportional to the total creatine (Cr + PCr) concentration, used as an internal reference, which was supposed to be 7.5 mmol/kg_{ww}.

A jMRUI window displaying the QUEST quantitation results, obtained for an *in vivo* rat brain signal acquired from the striatum and thalamus region, using an *in vitro* basis set, is shown in Figure 2. Ten of the eleven metabolites were reliably quantitated. The background signal (dashed line) is well modelled; both lipid resonances (0.9 and 1.3ppm) and the three principal resonances of macromolecules (around 2ppm, 3ppm and 3.9ppm) are well identified. The CRBs, were found in the range of 1% to 20% for all ten metabolites of the fifteen signals. An estimate was considered as relevant when the corresponding CRB was found below 15% of the estimate. In both approaches, quantitation of weakly represented metabolites, such as Lac, is not significant.

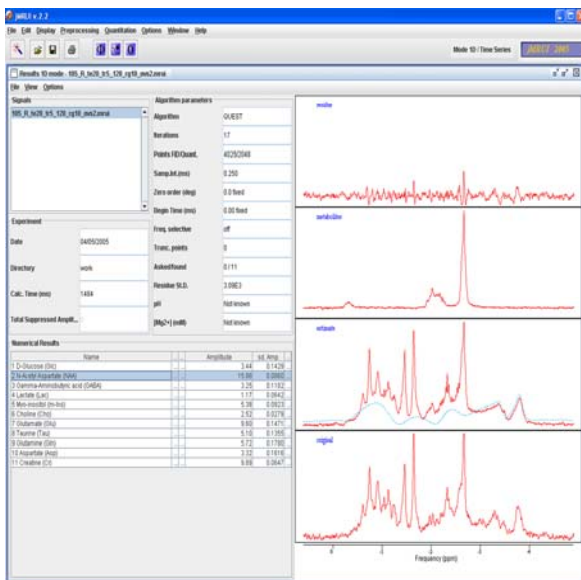


Figure 2: jMRUI QUEST Quantitation result window. From bottom to top, original spectrum of an *in vivo* rat brain acquired at 7T from the striatum and thalamus region using a PRESS sequence with an echo-time of 20ms; estimated spectrum and background (dashed line) using QUEST and *in vitro* metabolite basis set signals; selected metabolite (NAA) spectrum; and residue.

The mean values (in mmol/kg_{ww}) and the corresponding standard deviations of the estimated metabolite concentrations, obtained from the fifteen signals of different animals, were computed from the relevant estimates, see Figure 3 and Table I. The standard deviations reveal the inter-individual metabolite differences. The results obtained with the two metabolite basis sets are of comparable quality. As can be seen in Figure 3, the mean values of the estimated metabolite concentrations obtained using the simulated basis set are slightly higher than those obtained using the *in vitro* metabolite basis set. This could be explained by the fact that we used the same *in vitro* T_2 relaxation time value to correct the simulated basis set for *in vitro* T_2 effects, despite the fact that it is known that the metabolites other than NAA, Cho and Cr have smaller *in vitro* T_2 relaxation time values. The standard deviations obtained are similar for the two metabolite basis sets.

We also compared our estimated metabolite concentrations with the values from the literature (see Table I). The latter were estimated from biochemical rat brain assays [13, 14] and from quantitation of *in vivo* rat brain spectra using LC-Model [13, 14]. Our results are in good agreement with the published values.

In vitro metabolite basis set vs. simulated basis set

In summary, two approaches invoking prior knowledge in the fitting algorithm QUEST were compared. Both approaches present advantages and drawbacks.

For the *in vitro* basis set:

- + The experimental conditions are automatically taken into account.
- + The metabolite concentration estimates are automatically and partially compensated for the spin-spin relaxation effects.
- A new basis set must be acquired for any new experimental protocol.
- Tedious and time consuming experimental work is needed for acquiring signals of *in vitro* metabolite solutions.
- The basis set signals contain noise.

For the *simulated* basis set:

- + The basis set can easily and quickly be simulated for any experimental protocol.
- The metabolite concentration estimates should be compensated for the spin-spin relaxation effects, if the simulation program does not accommodate them during the simulated acquisition sequence. The transverse relaxation times of each metabolite must be previously known or measured.

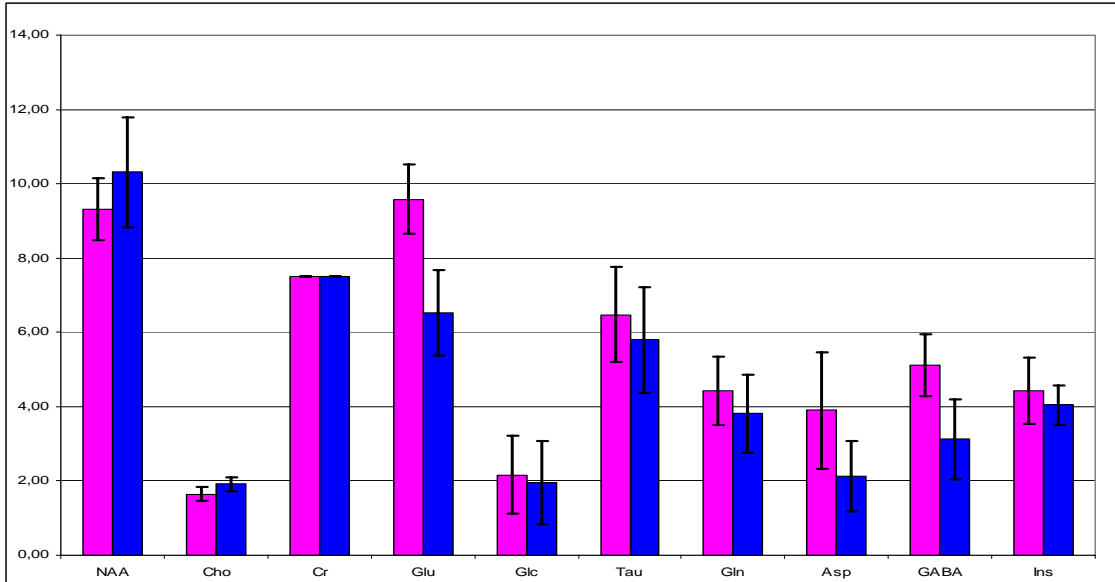


Figure 3: Mean values and corresponding standard deviations of the metabolite concentration estimates obtained from fifteen signals of eight rats, quantitated using QUEST and an *in vitro* metabolite basis-set (blue bars), and theoretical quantum mechanically simulated basis-set (pink bars). Cr was used as reference and consequently has no error bar.

Metabolite	This study <i>In vitro</i> basis set (Mean ±SD) mmol/kg _{ww}	This study Simulated basis set (Mean ±SD) mmol/kg _{ww}	Literature	
			Biochemical rat assays mmol/kg _{ww}	<i>In vivo</i> rat brain mmol/kg _{ww}
NAA	10.31±1.48	9.31± 0.84	4.7-9.7	8.3±0.5; 7.6-10
Cho	1.92±0.18	1.65 ± 0.18	-	0.7±0.1; 1.1
tCr (Cr+PCr)*	7.5	7.5	8.5-9.7	7.78; 8.3-9
Ins	4.04±0.54	4.43±0.88	-	4.08±0.5; 3.7
Tau	5.80±1.42	6.48±1.29	1.66-6.6	4.25±0.59; 8.1-10.8
Glu	6.53±1.15	9.57±0.99	7.4-12.5	8.67±0.7; 8.7-11.2
Gln	3.82±1.05	4.43±0.91	2.1-5.6	1.25±0.3; 2.8-3.5
Glc	1.98±1.11	2.17±1.06	0.96	2.7; 3.5±0.4
Asp	2.12±0.95	3.90±1.56	1.5-3.8	1.43±0.4; 1.2-2.4
GABA	3.12±1.07	5.12±0.83	0.8-2.3	1.1±0.25; 1.1

Table I: *In vivo* rat brain metabolite concentration estimates and confidence intervals estimated from fifteen signals of eight rats. For comparison, published metabolite concentrations from biochemical rat brain assays [13, 14] and *in vivo* rat brains [13, 14] are also displayed. *Only Cr was included in the metabolite basis set.

IV. CONCLUSIONS

- The striatum and thalamus region from healthy rat brains was investigated at 7Tesla.
- Metabolites were well identified and successfully quantitated using QUEST. The reliability of the reported concentration estimates of the main

metabolites was assessed using the Cramér-Rao lower bounds.

- Influence of *in vitro* and simulated metabolite basis set on QUEST quantitation results, were compared.
- The concentration estimates using the two basis sets are not significantly different and are in good agreement with the values from the literature.

ACKNOWLEDGMENT

We thank the ANIMAGE platform, Lyon, France, for giving us free access to the 7T MRI system.

REFERENCES

- [1] H. Ratiney, Y. Coenradie, S. Cavassila, D. van Ormondt, D. Graveron-Demilly, "Time-domain quantitation of ^1H short echo-time signals: background accommodation", *MAGMA*, vol. 16, pp. 284-296, 2004.
- [2] H. Ratiney, M. Sdika, Y. Coenradie, S. Cavassila, D. van Ormondt, D. Graveron-Demilly, "Time-domain semi-parametric estimation based on a metabolite basis set", *NMR Biomedicine*, vol. 18, pp. 1-13, 2005.
- [3] S.W. Provencher, "Estimation of metabolite concentrations from localized in vivo proton NMR spectra", *Magn. Reson. Med.*, vol. 30, pp. 672-679, 1993.
- [4] K. Young, V. Govindaraju, B.J. Soher, A.A. Maudsley, "Automated spectral analysis I: formation of a prior information by spectral simulation", *Magn. Reson. Med.*, vol. 40, pp. 812-815, 1998
- [5] R. Gruetter, "Automatic, localized in vivo adjustment of all first- and second- order shim coils", *Magn. Reson. Med.*, vol. 29, pp. 804-811, 1993.
- [6] I. Tkac, Z. Starcuk, I. Y. Choi, R. Gruetter, "In vivo ^1H NMR spectroscopy of rat brain at 1ms echo time ", *Magn. Reson. Med.*, vol. 41, pp. 649-656, 1999.
- [7] S. Cavassila, S. Deval, C. Huegen, D. van Ormondt, D. Graveron-Demilly, "Cramer-Rao bounds: an evaluation tool for quantitation", *NMR Biomedicine*, vol. 14, pp. 278-283, 2001.
- [8] D. Graveron-Demilly, A. Diop, A. Briguet, B. Fenet, "Product-operator algebra for strongly coupled spin systems", *J. Magn. Reson.*, vol. A101, pp. 233-239, 1993.
- [9] V. Govindaraju, K. Young, A.A. Maudsley, "Proton NMR chemical shifts and coupling constants for brain metabolites", *NMR Biomedicine*, vol. 13, pp. 129-153, 2000.
- [10] C. Cudalbu, S. Cavassila, D. Grenier, H. Ratiney, A. Briguet, D. Graveron-Demilly, *Book of Abstracts ESMRMB 2004 (Copenhagen)*, *MAGMA*, vol. 17, supp. 1, pp. 337, 2004.
- [11] <http://www.mrui.uab.es/mrui/>
- [12] A. Naressi, C. Couturier, J. M. Devos, M. Janssen, C. Mangeat, R. de Beer, D. Graveron-Demilly, "Java-based Graphical User Interface for the MRUI Quantitation " *MAGMA*, vol. 12, pp. 141-152, 2001.
- [13] J.Pfeuffer, I.Tkac, S.Provencher, R.Gruetter, "Toward an in Vivo Neurochemical Profile: Quantification of 18 Metabolites in Short Echo-Time ^1H Spectra of the rat Brain", *J. Magn. Reson.*, vol. 141, pp. 104-120, 1999.
- [14] I. Tkac, R. Rao, M. K. Georgieff, R. Gruetter, "Developmental and regional changes in the Neurochemical Profile of the Rat Brain Determined by In Vivo ^1H NMR Spectroscopy", *Magn. Reson. Med.*, vol. 50, pp. 24-32, 2003.