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Single-voxel ^1H spectroscopy in the human hippocampus at 3 T using the LASER sequence: characterization of neurochemical profile and reproducibility

Najib Allaïli^{1,2,3,4,5,6}, Romain Valabrègue^{1,2,3,4}, Edward J. Auerbach⁷, Vincent Guillemot^{2,3,4,8}, Lydia Yahia-Cherif^{1,2,3,4}, Eric Bardinet^{1,2,3,4}, Maritza Jabourian⁹, Philippe Fossati¹⁰, Stéphane Lehericy^{1,2,3,4,11}, and Małgorzata Marjańska⁷

¹ Institut du cerveau et de la Moelle épinière – ICM, Centre de Neurolmagerie de Recherche – CENIR, Paris, France

² Inserm U 1127, Paris, France

³ CNRS UMR 7225, Paris, France

⁴ Sorbonne Universités, UPMC Univ Paris 06 UMR S 1127, Paris, France

⁵ Université Paris Diderot, Sorbonne Paris Cité, Paris, F-75013, France

⁶ APHP – Département de Psychiatrie et de Médecine Addictologique, Groupe Hospitalier Saint-Louis – Lariboisière – Fernand-Widal, Paris, France

⁷ Center for Magnetic Resonance Research and Department of Radiology, University of Minnesota, Minneapolis, USA

⁸ ICM, Plate-forme de Bio-informatique/Biostatistique, F-75013, Paris, France

⁹ Servier, France

¹⁰ Department of Psychiatry, Groupe Hospitalier Pitié-Salpêtrière, Paris, France

¹¹ Department of Neuroradiology, Groupe Hospitalier Pitié-Salpêtrière, Paris, France

Abstract

The hippocampus is crucial for long-term episodic memory and learning. It undergoes structural change in aging and is sensitive to neurodegenerative and psychiatric diseases. MRS studies have seldom been performed in the hippocampus due to technical challenges. The reproducibility of MRS in hippocampus has not been evaluated at 3 T. The purpose of the present study was to quantify the concentration of metabolites in a small voxel placed in the hippocampus and evaluate the reproducibility of the quantification. Spectra were measured in a 2.4 mL voxel placed in the left hippocampus covering the body and most of the tail of the structure in ten healthy subjects across three different sessions and quantified using LCModel. High quality spectra were obtained which allowed a reliable quantification of ten metabolites including glutamate and glutamine. Reproducibility of MRS was evaluated with coefficient of variation, standard errors of

measurement, and intraclass correlation coefficients. All of these measures showed improvement with increased number of averages. A change of less than 5% in concentration of *N*-acetylaspartate, choline containing compounds and total creatine and of less than 10% in concentration of *myo*-inositol and sum of glutamate and glutamine can be confidently detected between two measurements in a group of twenty subjects. A reliable and reproducible neurochemical profile of the human hippocampus was obtained using MRS at 3 T in a small hippocampal volume.

Graphical Abstract

High-quality spectra which allowed reliable quantification of ten metabolites were measured in a 2.4 mL voxel placed in the left hippocampus in ten healthy subjects. The reproducibility in metabolite concentrations improved with increased number of averages. The reproducibility achieved in this study shows that a change of less than 10% in concentration can be confidently detected for five major metabolites in a group of twenty subjects.

Keywords

MRS reproducibility; hippocampus; 3 T; CV; ICC; sensitivity; glutamate; glutamine

Introduction

The hippocampal formation is a key structure in the brain that is crucial for long-term episodic memory (1). The hippocampus also has an important role in neurogenesis associated with learning and memory in the adult brain (2). The hippocampus undergoes structural changes with aging (3) and is sensitive to numerous neurodegenerative and psychiatric diseases, from Alzheimer's disease (AD) to schizophrenia and depression (3). Reduced volume observed with structural MRI is a hallmark of AD (4), but was also found in other neurodegenerative diseases, such as fronto-temporal dementia (5) and Parkinson's disease (6), and in depressed patients (7,8).

Proton (^1H) magnetic resonance spectroscopy (MRS) is a unique technique that allows the detection and quantification of brain metabolites *in vivo*. Quantification of specific metabolites has been used to detect damage in brain structures (9-12), follow disease progression (13), as well as to study the effect of medication (14). Metabolites provide

information about various biological processes. For instance, *N*-acetylaspartate (NAA) is considered to be a marker of neuronal density and health, choline-containing compounds (tCho) are markers of demyelination and cell proliferation, and *myo*-inositol (mIns) is thought to be a marker for osmotic stress or astrogliosis. Quantification of glutamate (Glu) and glutamine (Gln) levels in different diseases and in response to treatment can provide insights into changes in glutamatergic neurotransmission, as suggested by preclinical studies in depression (15,16).

MRS studies have seldom been performed in the hippocampal formation due to technical challenges associated with that brain region (17-23). The hippocampus is adjacent to structures such as the petrous bone and sinuses, which cause poor homogeneity of the B_0 magnetic field and large susceptibility gradients resulting in broader linewidths and low spectral resolution relative to other brain regions. Another major impediment to MRS in the hippocampus is the small size of the structure and its location in the midbrain, resulting in poor signal-to-noise ratio (SNR). Low spectral resolution and SNR have a major effect on spectral quality and consequently on data reliability and reproducibility. Additionally, difficulties in voxel positioning on repeat scans add to uncertainties in data reproducibility. Although few studies have investigated the reproducibility of MRS in the hippocampus (18-20,22,23), most have used large voxels compared to the size of the hippocampus (4 to 6.2 mL) (18,20,22,23), and none have been conducted at 3 T.

The aims of the current study were 1) to assess the feasibility of reliably measuring metabolite concentrations in the hippocampus with optimal voxel size of 2.4 mL at a clinically relevant field strength of 3 T; 2) to characterize the quantifiable neurochemical profile; 3) to quantify the reproducibility of repeated spectroscopic measurements in this region; and 4) to determine the effect size for future clinical studies.

Experimental

Subjects

Healthy volunteers (n = 10, 6 M, 4 F, mean age $\pm$ SD: 28 ± 4 years) were studied after giving written informed consent according to the procedures approved by the Ethics Committee of the Pitié-Salpêtrière Hospital.

Study protocol

Each subject was scanned during three different sessions that took place on three consecutive days. For each subject, scans were obtained at the same time of the day to minimize any possible daily variation in metabolite concentrations. Each session lasted one hour, including positioning in the magnet and MR acquisition.

MR acquisition

All MR experiments were performed on a 3 T whole-body system (MAGNETOM Trio, A Tim System; Siemens, Erlangen, Germany). Radiofrequency transmission was performed with a whole-body coil, and signal was received with a 12-channel receive-only phased-array head coil.

Three-dimensional (3D) T_1 -weighted magnetization prepared rapid acquisition gradient echo (MPRAGE) images were acquired for voxel positioning and tissue segmentation ($T_R = 2.3$ s, $T_E = 4.18$ ms, $T_1 = 900$ ms, flip angle = 9° , number of slices = 176, matrix size = 256×256 , field of view (FOV) = $256 \text{ mm} \times 256 \text{ mm}$, slice thickness = 1 mm) during each session.

All spectra were acquired using a localization by adiabatic selective refocusing (LASER) sequence (24) from 2.4 mL voxel placed in the left hippocampus (Figure 1A). After water suppression was performed with variable pulse power and optimized relaxation delays (VAPOR) (25), all resonances were excited by using a nonselective 5.12 ms adiabatic half-passage pulse which was numerically optimized to satisfy the adiabatic condition for wide range of B_1 and frequency using a previously described numerical method (26). Three-dimensional localization was then performed with a pair of offset-independent adiabatic pulses (HS1, pulse length of 8.5 ms, bandwidth of 2.35 kHz) (24,27) in each dimension. Each free induction decay (FID) was acquired with 2048 complex points and a spectral width of 1.2 kHz. FIDs were stored separately in memory. The T_R was 3 s, $T_E = 65$ ms. Spectra were acquired in three blocks of 256 averages, with each acquisition preceded with 4 preparation scans (discarded) and a reference scan with no water suppression that was used for calculating the coil combination parameters. A separate water reference was also acquired ($T_R = 15$ s, number of averages (N_A) = 4) for eddy current correction and quantification. The total acquisition time was 42 minutes. Each voxel measurement began with an adjustment of the first- and second-order shims using FASTMAP (fast automatic shimming technique by mapping along projections) with echo-planar imaging readout (28) over the voxel only.

Six of the volunteers were scanned additionally to obtain T_2 relaxation times for tNAA, tCr, and tCho in the hippocampus. T_E was extended by adding delays around the last AFP pulse in the LASER sequence, and spectra were collected from an 8 mL voxel centered on the left hippocampus at seven T_{ES} : 65, 95, 125, 155, 185, 275 and 400 ms. To compensate for SNR losses due to longer T_E , more averages were acquired at longer T_E (128 averages for T_E 125 ms, 192 averages for T_E of 155 and 185 ms, and 256 averages for T_E 275).

MRS voxel placement

Each subject's head was positioned in the scanner using the laser beam, and foam wedges were placed on both sides of the head to immobilize it in the coil. The MRS voxel (Figure 1A) was angulated along the anterior-posterior hippocampal axis and positioned in the following way: (1) left-right – 12 mm; (2) anterior-posterior – 25 mm with the anterior part of the voxel starting right after the uncus in order to ensure that the hippocampal head is not included; (3) superior-inferior – 8 mm. Images from the first session showing the voxel location were recorded and used for voxel placement in subsequent sessions.

MR data processing and quantification

FIDs were frequency and phase corrected and summed to generate four sets of data from each session with number of averages equal to 256, 384, 512, and 768. All spectra underwent eddy current correction and automatic adjustment of the zero-order phase. Additionally, a spectrum with 2304 averages was generated for each subject from all the

data acquired during three sessions. A total of 13 spectra were generated per subject. Data from a single session from one subject was excluded due to severe artifacts. A total of 126 spectra underwent analysis.

All spectra were analyzed with LCModel 6.1-4A (29) (Stephen Provencher, Inc., Oakville, Ontario, Canada), with the basis set generated using in-house programs based on the density matrix formalism (30) in Matlab (The MathWorks, Inc., Natick, MA, USA) using the known chemical shifts and J -couplings (31). Simulated spectra of the following 17 metabolites were included in the basis set: ascorbate (Asc), aspartate (Asp), creatine (Cr), γ -aminobutyric acid (GABA), glucose (Glc), Glu, Gln, glycerophosphorylcholine (GPC), glutathione (GSH), lactate (Lac), mIns, N -acetylaspartate (NAA), N -acetylaspartylglutamate (NAAG), phosphocreatine (PCr), phosphorylcholine (PCho), *scyllo*-inositol (sIns), and taurine (Tau). Experimentally measured metabolite-nulled macromolecules (MM) spectra from the occipital region of five additional subjects ($T_R = 3$ s, $T_E = 65$ ms, $T_1 = 840$ ms) were also included in the basis set as it has not been demonstrated that the MM pattern is brain region dependent to the extent that it impacts metabolite quantification (32,33). No zero-filling or line-broadening were applied to the *in vivo* data prior to the analysis. The LCModel fitting was performed over the spectral range from 0.5 to 4.2 ppm.

Quantification was performed using an unsuppressed water signal obtained from the same voxel. Concentrations were corrected for cerebrospinal fluid (CSF) content. The tissue composition was measured using 3D MPRAGE images, which were segmented into gray matter (GM), white matter (WM) and CSF using SPM8 software (<http://www.fil.ion.ucl.ac.uk/spm>). The fractional volumes of GM, WM, and CSF were obtained by averaging GM, WM, and CSF segmented images over the spectroscopic voxel. The relative densities of MR-visible water for GM, white matter WM, and CSF were assumed to be 0.78, 0.65, and 0.97, respectively (34). For the calculation of attenuation factors, T_2 of water and T_1 of metabolites were taken from literature (35,36), and T_2 for tNAA, tCr, and tCho were obtained in this study. The T_2 of the metabolites that were not measured were assumed to be similar to that of tCr at 3.03 ppm. To correct for the Carr-Purcell effects, T_2 of water and T_2 of metabolites were multiplied by a factor of 1.5 (37-39). The T_1 relaxation of water was not taken into consideration since unsuppressed water spectra were obtained with T_R of 15 s.

Only metabolites quantified with Cramér-Rao lower bounds (CRLB) $\leq 50\%$ in at least half of the spectra (39) with a given number of averages were included in the neurochemical profile. If the covariance between two metabolites was consistently high (correlation coefficient below -0.5), as found for Cr and PCr, their sum was reported rather than their individual values.

T_2 fitting—The T_2 relaxation times of metabolites were determined by fitting the natural logarithm of the relative concentrations obtained from the LCModel analysis as a function of T_E using a linear regression algorithm. The fitting was performed only for metabolites for which CRLB were below 20% at all T_E s.

Statistical analysis

All statistical analyses were performed using R software version 2.13.2 (R Foundation for Statistical Computing, Vienna; <http://www.r-project.org>).

The reliability of MRS measurements for tNAA, tCho, tCr, mIns, and Glx was estimated using the coefficients of variation (CV), the standard errors of measurement (SEM), and the intraclass correlation coefficients (ICC) over three sessions in nine subjects. Data from one subject was removed from these calculations because data from one session had severe artifacts which were not present in any of the other spectra.

CV were calculated as the ratio of the standard deviation (SD) to the mean.

The ICC and SEM were obtained based on following linear model (40):

$$M_{ij} = \mu + Sub_i + Scan_j + e_{ij}$$

where M_{ij} is the measurement in subject i (Sub_i) scanner j ($Scan_j$), μ is the mean and e_{ij} a residual error term. The variance components of the linear model were estimated with a restricted maximum likelihood (REML) approach in order to obtain unbiased positive values.

Three variance components were estimated: 1) between subject variance, σ_b^2 ; 2) within subject variance, σ_w^2 ; 3) residual variance, σ_r^2 . Given these variance components, intraclass correlation coefficients (ICCs), measuring absolute test-retest reliability, were calculated using following equation:

$$ICC = \frac{\sigma_b^2}{\sigma_b^2 + \sigma_w^2 + \sigma_r^2}$$

The SEMs were used to assess in a similar way the reliability and were defined as:

$$SEM = \sqrt{(\sigma_w^2 + \sigma_r^2)} \times \sqrt{1 - \alpha},$$

where α was estimated using the Cronbach function from 'psy' in R. The α in Cronbach's statistic is used to measure reliability of several raters; in the case of this study, the scanning sessions are considered as different raters.

Sensitivity, defined as the range of 95% confidence interval on the mean of n measurements, under the assumption of known measurement variance (41,42), normalized by the mean were calculated using following equation:

$$\text{sensitivity} = 1.96 \times \sqrt{2} \times \frac{CV}{\sqrt{n}}$$

Median CV values from 9 subjects were used to determine the sensitivity for observing a change in a longitudinal study in a cohort of 20 individuals, a typical number of subjects in clinical studies.

Results

Voxel placement

The MRS voxel was consistently placed in the same anatomical location containing hippocampus, parahippocampal gyrus, perihippocampal WM, and CSF. The voxel was composed of $63 \pm 8\%$ GM, $31 \pm 8\%$ WM, and $6 \pm 3\%$ CSF, as determined by the SPM8 tissue segmentation, with no significant differences between three sessions.

Data quality

High-quality spectra without any contamination from signals outside the voxel such as lipids and with effective water suppression as evidenced by a flat baseline were obtained in 29 sessions out of 30 (Figure 1B). The full width at half maximum (FWHM) values as reported by LCModel, which provide a rough estimate of the linewidth in the *in vivo* spectra, were 4.1 ± 0.7 Hz for spectra acquired in session 1, 4.5 ± 0.3 Hz in session 2 and 4.3 ± 0.7 Hz in session 3, with no significant difference between the three sessions. The SNR obtained from LCModel, which is defined as the ratio of the maximum signal in the spectrum minus the baseline to twice the root-mean-square of the residual, were 11.3 ± 0.9 for spectra with 256 averages in session 1, 10.9 ± 1.3 in session 2, and 10.9 ± 1.9 in session 3, with no significant difference between the three sessions. In addition, the SNR obtained from LCModel were 11 ± 1 , 14 ± 1 , 15 ± 2 , 18 ± 2 , 30 ± 2 for spectra with $N_A = 256, 384, 512, 768$, and 2304 , respectively.

T_2 relaxation times

The T_2 values were obtained for tNAA (2.01 ppm), tCho (3.2 ppm) and tCr_{CH3} (3.03 ppm) as those were the only metabolites for which CRLB were below 20% at all T_E s. Excellent fits were obtained for all data sets (Figure 2) ($R = -0.98 \pm 0.02$, -0.97 ± 0.03 , -0.995 ± 0.002 for tNAA, tCho and tCr_{CH3}, respectively). Measured T_2 values were 324 ± 29 ms, 312 ± 65 ms and 180 ± 20 ms for tNAA, tCho, and tCr_{CH3}, respectively.

Neurochemical profile

Table 1 and Figure 3 report the concentrations of metabolites that were quantified for N_A ranging from 256 to 2304 in a 2.4-mL voxel in the left hippocampus. From spectra with 256 averages, 10 metabolites were quantified with CRLB < 50% in at least 50% of the cases. tNAA, tCho, tCr, and mIns were quantified with mean CRLB < 5% while Glx, Glu, Glc + Tau, GSH + Asc were quantified with mean CRLB < 25%. The highest CRLB, < 35%, were obtained for sIns and Gln. Spectra with 512 averages allowed quantification of Lac which has a distinct spectral pattern at this T_E due to *J*-modulation. Using spectra with 768 averages, Glu, Gln and Lac were quantified in 21 out of 29 spectra with mean CRLB around 7, 27, and 30%, respectively. Asp was only quantified in the summed spectrum from all three sessions ($N_A = 2304$). Mean CRLB improved for all metabolites with increased number of averages as shown in Figure 4. Additionally, the number of spectra in which

lower concentration metabolites, such as Gln, were quantified increased with the number of averages. Gln was quantified in 59% of spectra with $N_A = 256$, 69% with $N_A = 384$, 72% with $N_A = 512$, and 76% with $N_A = 768$. For all metabolites reported in Table 1 except Lac, the obtained concentrations did not differ significantly with number of averages.

Reproducibility

Reproducibility data for five metabolite concentrations for 9 subjects and three sessions are reported in Table 2. The reproducibility for Glx rather than Glu and Gln was reported since Glu and Gln were not quantified in all spectra. CVs for all five metabolites improved with increased N_A . The most significant improvement was observed going from 256 averages to 512 averages. Additional improvement was observed going from 512 averages to 768 averages, but it was not significant. For tNAA, tCho, tCr, and mIns, reproducibility was already very good for $N_A = 256$ with median CVs < 10%, and improved to < 6% with $N_A = 512$. The poorest reproducibility was observed for Glx, for which CVs close to 10% were obtained with $N_A = 512$ and 768.

The improvement in reproducibility of metabolite concentrations with increased N_A was observed for two other estimators of measurement reproducibility, SEM and ICC (Table 2). Although the decrease in both CV and SEM is striking and illustrates well the effect of N_A on measuring metabolite concentrations, ICC complement nicely these measures by providing a relative variability estimator scaled between 0 and 1. The use of the three estimators together is highly recommended in similar experiments. While SEM and CV provide an absolute index of reliability, ICC provides a relative ratio of subject variability to total variability allowing thus, a better comparison of reproducibility between metabolites.

Sensitivity to change

Using obtained CVs, sensitivity calculations were performed to determine the changes that could be detected in a group of 20 subjects between two independent scans (Table 3). For $N_A = 256$, a change of less than 10% in concentration can be confidently detected between two measurements for all five metabolites, tNAA, tCho, tCr, mIns, and Glx, in a cohort of 20 subjects. For three main metabolites, tNAA, tCho, and tCr, less than 5% change in concentration can be detected. The minimum sample size needed to reliably detect changes of 10% in Glx concentration in the hippocampus at 3 T is 20 subjects.

Discussion

This study demonstrated that high-quality, single-voxel spectra can be obtained from a 2.4 mL voxel placed in the hippocampus at 3 T using a clinical system. Spectra were obtained using a fully-adiabatic LASER sequence (24) with T_E of 65 ms, which offers excellent localization performance without the need for OVS and reduced chemical-shift displacement error (~5% /ppm/direction). Additionally, at this longer T_E , the contribution from the macromolecule baseline is reduced, but the effect on metabolites is mitigated by reduced J -modulation and increased apparent T_2 of metabolites (37,38,43), characteristic of the LASER sequence. High spectral resolution and narrow linewidths were achieved with efficient B_0 shimming.

Almost all previous studies in the hippocampus have only reported the concentrations or concentration ratios of tNAA, tCr, tCho, and mIns using MRS measured at short or long T_E (17-20,22,23). The spectral quality achieved in this study using 256 averages allowed the quantification of ten metabolites using LCModel analysis. Seven metabolites were quantified with average CRLB of less than 20%, indicating that these were the most robustly quantified neurochemicals. As expected, more metabolites were quantified and with better precision with higher numbers of averages. Generally, standard deviations and CRLB decreased as number of averages increased, while estimated metabolite concentrations remained relatively constant. The concentrations of metabolites quantified from these spectra were consistent with previously reported literature values (17-20). The quantification was performed by taking into consideration the CSF content, tissue composition, relative density of MR-visible water, and the relaxation times of water and metabolites. The T_2 values of metabolites measured were similar to those previously reported at the similar field strength (44,45).

In our study, multi-subject CV of absolute concentration for five major metabolites ranged from 5.7 to 10.6% with the lowest N_A and from 3.7 to 9.9% with the highest N_A . The highest CVs were observed for mIns and Glx (9.6% and 10.6%). These values were lower than previously reported values from other studies performed in the hippocampus. In studies performed at 1.5 T with larger voxel size (~6 mL), the CVs for tNAA, tCr, tCho and mIns ranged from 7.4% to 26% (18,23). The reported CVs for Glu and Gln were very high (18). Another study at 1.5 T, which used a voxel size comparable to this study, 2 mL, reported CV ranging from 8.8% to 18% for the four main metabolites, and 19.4 % for Glx (19). In a study performed at 4 T with a 4 mL voxel size, both intra-individual and inter-individual subject CVs were reported for tNAA, tCr, tCho and ranged from 13.9% to 18.1%. Additionally, the intra-individual CVs were reported for mIns, Glu, and Glx and ranged from 11% for mIns to 21% for Glu. One study optimized for the detection of Glu at 3 T reported CVs on the order of 10% for Glu from a 12 mL voxel placed in the hippocampus (44).

ICC showed high reliability (0.75 for mIns and tCho) only for 768 averages. This is due primarily to ICC sensitivity to between-subject variations such as systematic differences in spectral quality and individual anatomical differences (size and shape of the hippocampus), which could not be reduced because of the small number of subjects. Low ICC indicates that between-subject variance is low compared to all other sources of variance. The between-subject variance is, in principle, related to real differences in metabolite concentrations among subjects. Accordingly, given a certain level of noise, low ICC would be expected when subject-to-subject differences in the measured metabolite concentrations are small. This could be the case for tCr measured in the healthy subject cohort, since tCr concentration is known to be stable across individuals and is for this reason often used as an MRS reference for relative metabolite quantification (46). For better assessment of reproducibility, especially for healthy subjects, ICC could be complemented with CV and SEM.

The results in this study compared well with the results obtained at 3 T for another small (1.8 to 2.6 mL) and challenging region, amygdala (47). In amygdala, between-individual CVs with spatial correction ranged from 8.9 to 17.5% for tNAA, tCr, tCho, mIns and Glx,

while in this study, CVs ranged from 5.7 to 10.6%. Similarly to this study, the highest CVs were observed for mIns and Glx. However, in this study, highest CVs were observed for Glx, while for amygdala highest CVs were observed for mIns. Additionally, ICC ranged from 0.28 to 0.70 for amygdala while in this study, ICC ranged from 0.1 to 0.35 for the same N_A .

The measurement precision obtained in this study would allow detection of 5% changes for tNAA, tCr, and tCho, 6% changes for mIns and 10% changes for Glx in a group of 20 subjects in a small voxel placed in the hippocampus. The feasibility of detecting this level of differences in a group of 20 subjects is clinically relevant as larger than 10% differences in the concentration of NAA have been reported in patients with AD (11,13), temporal lobe epilepsy (48,49) and schizophrenia (50) and in the concentration of Glx in patients with AD (51,52).

Conclusion

This study demonstrates that high-quality spectra can be obtained from a small voxel placed in the hippocampus at 3 T. The reliable quantification of 10 metabolites including Glu and Gln was possible from spectra with 256 averages. The precision with which metabolites were quantified increased with number of averages. The results of this study demonstrate high reproducibility for five major metabolites and an ability to reliably detect changes in metabolite concentrations in longitudinal studies.

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List of abbreviations

AD	Alzheimer's disease
CRLB	Cramér-Rao Lower Bounds
CSF	cerebrospinal fluid
CV	coefficient of variation
FID	free induction decay
Gln	glutamine
Glu	glutamate
GM	gray matter
ICC	intraclass correlation coefficient
LASER	localization by adiabatic selective refocusing
N_A	number of averages

mIns	<i>myo</i> -inositol
SEM	standard error of measurement
SNR	signal-to-noise ratio
SD	standard deviation
tNAA	total NAA, <i>N</i> -acetylaspartate + <i>N</i> -acetylaspartylglutamate
tCho	total choline, choline-containing compounds
tCr	total creatine, creatine + phosphocreatine
VAPOR	variable pulse power and optimized relaxation delays
WM	white matter

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Figure 1. Voxel placement and representative spectra

(a) The location and size of the 2.4-mL voxel ($12 \times 8 \times 25 \text{ mm}^3$) placed in the left hippocampus shown on T_1 -weighted images. (b) LCMoDel quantification of representative spectra obtained at 3 T with the LASER sequence from the hippocampus voxel, with N_A ranging from 256 to 2304. $T_R = 3 \text{ s}$, $T_E = 65 \text{ ms}$. No line broadening was applied. The MM profile and its contribution to the fit of spectrum with $N_A = 2304$ shown.

Figure 2. T_2 fits

Individual fits of the experimentally measured data in an 8-mL voxel in the left hippocampus for the tNAA, tCho and methyl protons of tCr. The amplitudes of the natural logarithm of all data sets were normalized by setting the first T_E point to unity. Error bars represent the standard deviation between subjects.

Figure 3. Concentrations of brain metabolites measured at 3 T in the human hippocampus from Table 1 summarized as box plots

The boxes show the 25th percentile, the 50th percentile, and the 75th percentile of the data with “whiskers” extending out 1.5 times the inter-quartile range. For a given N_A , metabolite is plotted if it was quantified with CRLB < 50% in 50% of the spectra.

Figure 4. Average CRLB from Table 1

The average CRLB are plotted for all metabolites in Table 1 except Asp as a function of N_A .

Average concentrations and CRLBs (with SD) of brain metabolites measured at 3 T in the human hippocampus (10 subjects) with N_A ranging from 256 to 2304 and quantified using LCModel.

Table 1

Metabolite	Concentration (mM)					CRLB(%)					
	N_A	256	384	512	768	2304	256	384	512	768	2304
tNAA		10.0 ± 0.9	10.0 ± 1.1	10.0 ± 1.0	9.8 ± 0.8	9.8 ± 0.8	2.8 ± 0.6	2.2 ± 0.4	2.1 ± 0.3	2.0 ± 0.2	1.1 ± 0.3
tCr		7.9 ± 0.9	8.0 ± 1.0	7.9 ± 0.9	7.8 ± 0.7	7.8 ± 0.6	3.4 ± 0.6	2.8 ± 0.4	2.5 ± 0.5	2.1 ± 0.3	1.6 ± 0.5
tCho		2.2 ± 0.2	2.2 ± 0.2	2.2 ± 0.2	2.2 ± 0.2	2.2 ± 0.2	4.1 ± 0.7	3.4 ± 0.5	3.1 ± 0.3	2.9 ± 0.4	2.0 ± 0.0
mIns		8.3 ± 0.9	8.2 ± 0.9	8.3 ± 0.9	8.1 ± 0.9	7.8 ± 0.8	4.9 ± 1.0	4.2 ± 0.7	3.9 ± 0.5	3.4 ± 0.5	2.7 ± 0.5
Glu		7.8 ± 2.4	7.8 ± 1.7	8.0 ± 1.5	7.8 ± 1.3	8.1 ± 0.9	11 ± 2	10 ± 2	9 ± 1	7.5 ± 0.8	5.0 ± 0.5
Glx		10.0 ± 2.7	10.1 ± 2.1	10.4 ± 1.9	10.0 ± 1.9	10.1 ± 1.8	13 ± 3	11 ± 3	9 ± 2	9 ± 2	5.4 ± 0.8
Glc + Tau		4.0 ± 1.5	3.6 ± 1.1	3.5 ± 1.0	3.4 ± 1.0	3.3 ± 0.9	18 ± 8 [28]	17 ± 6	16 ± 4	15 ± 5	10 ± 2
GSH + Asc		3.0 ± 1.6	2.9 ± 1.3	2.5 ± 1.1	2.3 ± 1.2	2.0 ± 0.9	24 ± 10 [23]	19 ± 8 [24]	21 ± 9 [25]	20 ± 9 [26]	15 ± 5
sIns		0.5 ± 0.2	0.4 ± 0.2	0.4 ± 0.2	0.4 ± 0.2	0.4 ± 0.2	28 ± 11 [19]	23 ± 9 [22]	21 ± 7 [22]	20 ± 9 [24]	15 ± 8
Gln		3.3 ± 1.2	3.0 ± 1.1	3.0 ± 0.7	2.7 ± 0.9	2.5 ± 0.7	34 ± 10 [17]	31 ± 10 [20]	26 ± 6 [21]	27 ± 10 [22]	18 ± 6 [8]
Lac		-	-	0.7 ± 0.3	0.6 ± 0.2	0.5 ± 0.1	-	-	33 ± 10 [21]	30 ± 8 [21]	22 ± 6

29 spectra per N_A for $N_A < 2304$ (3 spectra per subject $\times$ 10 subjects minus one spectrum removed from one subject) and 9 spectra for $N_A = 2304$. For a given N_A , a metabolite is reported only if it was quantified with CRLB < 50% in more than 50% of the spectra. Concentrations with CRLB > 50% were classified as not detected and excluded from any analysis. For the metabolites which were not quantified in all spectra, the number of spectra in which they were quantified are listed in square brackets in CRLB section.

Reproducibility data for main metabolites quantified in the human hippocampus with different N_A .

Table 2

Metabolite	NA	Median CV (%)	SEM (%)	Variance					ICC
				between-subject (σ_b^2)	%	within-subject (σ_w^2)	%	residual (σ_r^2)	
tNAA	256	5.7	2.7	0.30	35	0.00	0	0.56	65
	384	4.2	3.3	0.31	25	0.02	1	0.93	74
	512	4.8	2.9	0.38	33	0.24	18	0.76	51
	768	4.1	1.8	0.42	57	0.00	0	0.32	43
tCho	256	7.0	1.1	0.03	33	0.009	9	0.06	59
	384	4.1	1.3	0.03	32	0.020	19	0.06	50
	512	4.6	1.2	0.02	26	0.012	15	0.05	59
	768	3.1	0.3	0.04	74	0.002	3	0.01	24
tCr	256	7.7	3.4	0.11	15	0.03	4	0.63	81
	384	6.0	5.1	0.37	28	0.16	10	0.96	61
	512	4.9	4.4	0.42	33	0.12	9	0.86	59
	768	4.7	2.0	0.19	38	0.00	0	0.31	62
mIns	256	9.6	11.5	0.29	26	0.39	25	0.85	51
	384	7.1	5.6	0.32	26	0.16	12	0.93	63
	512	5.6	4.2	0.50	35	0.15	10	0.92	55
	768	4.1	1.4	0.62	75	0.00	0	0.20	25
Glx	256	10.6	15.9	0.77	10	1.37	16	6.68	75
	384	13.1	8.4	0.80	17	0.38	8	3.60	75
	512	9.2	6.2	1.66	38	0.57	13	2.12	49
	768	9.9	4.4	2.15	56	0.00	0	1.71	44

CV, SEM, and ICC were obtained using data from 9 subjects and 3 sessions. Percent of total variance “%” for each variance component appears in the adjacent column to the right.

Table 3

Sensitivity to metabolite concentration change between two independent scans in a group of 20 subjects at a significance level of 0.05.

Metabolite	Sensitivity (%)				
N _A	256	384	512	768	
tNAA	3.5	3.4	3.4	2.5	
tCho	4.9	3.7	3.7	2.7	
tCr	4.9	3.9	3.2	2.9	
mIns	6.0	4.6	3.7	2.4	
Glx	9.6	8.2	7.0	5.8	