High-field localized $^1$H NMR spectroscopy in the anesthetized and in the awake monkey

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Abstract

Localized cerebral in vivo $^1$H NMR spectroscopy (MRS) was performed in the anesthetized as well as the awake monkey using a novel vertical 7 T/60 cm MR system. The increased sensitivity and spectral dispersion gained at high field enabled the quantification of up to 16 metabolites in 0.1- to 1-ml volumes. Quantification was accomplished by using simulations of 18 metabolite spectra and a macromolecule (MM) background spectrum consisting of 12 components. Major cerebral metabolites (concentrations $>3$ mM) such as glutamate (Glu), N-acetylaspartate (NAA), creatine (Cr)/phosphocreatine (PCr) and myo-inositol (Ins) were identified with an error below 3%; most other metabolites were quantified with errors in the order of 10%. Metabolite ratios were 1.39:1 for total NAA, 1.38:1 for glutamate (Glu)/glutamine (Gln) and 0.09:1 for cholines (Cho) relative to total Cr. Taurine (Tau) was detectable at concentrations lower than 1 mM, while lactate (Lac) remained below the detection limit. The spectral dispersion was sufficient to separate metabolites of similar spectral patterns, such as Gln and Glu, N-acetylaspartylglutamate (NAAG) and NAA, and PCr–Cr. MRS in the awake monkey required the development and refinement of acquisition and correction strategies to minimize magnetic susceptibility artifacts induced by respiration and movement of the mouth or body. Periods with major motion artifacts were rejected, while a frequency/phase correction was performed on the remaining single spectra before averaging. In resting periods, both spectral amplitude and line width, that is, the voxel shim, were unaffected permitting reliable measurements. The corrected spectra obtained from the awake monkey afforded the reliable detection of 6–10 cerebral metabolites of 1-ml volumes.

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1. Introduction

In vivo localized $^1$H MR spectroscopy is of great importance for neurobiology, as it can be set out to noninvasively investigate the bioenergetics, metabolism and neurochemistry of normal or diseased brains of animals ranging from mouse to human [1,2]. While in the past, most $^1$H NMR spectroscopy (MRS) investigations in vivo focused on detecting changes of signals from the methyl groups of N-acetylaspartate (NAA), total creatine [creatinine (Cr)+phosphocreatine (PCr)], choline (Cho) and lactate (Lac), an increasingly large number of current studies aim to detect an extended neurochemical profile and study its dynamic changes [3–5].

The sensitivity and spectral resolution afforded at high magnetic fields [6] made it possible to detect up to 18 brain metabolites by $^1$H MRS in the rat and mouse at 9.4 T [7,8]. The use of short echo times [9–11] minimizes $T_2$ signal losses and increases the contributions of coupled spin systems such as glutamate (Glu), glutamine (Gln), myo-inositol (Ins), glucose (Glc) and taurine (Tau). It also facilitates the detection of broad signals with short $T_2$, typically originating from macromolecules (MMs) and lipids [12–17]. The latter are of clinical importance. Indeed, several studies attempt to derive diagnostic information from the presence of mobile lipids and pathologically altered MMs by including them in the spectral evaluation model [18–21]. In other words,
quantification is attempted by including prior knowledge not only from metabolites of in vitro model $^1$H spectra, but also from the expected contributions of lipids and MMs using simulated $^1$H spectra, which partially or completely substitute in vitro and in vivo measurements [10,22]. MRS studies ($^1$H, $^{13}$C, $^{19}$F, $^{31}$P) were also reported in monkeys [23–34]. $^1$H MRS in the anesthetized monkey, mostly performed using clinical 1.5- or 3-T MR systems, quantified five major cerebral metabolic components: NAA+NAAAG, Glu+Gln, Cr+PCr, Ins and Cho [30,32]. To the best of our knowledge, no $^1$H MRS studies have been reported in the awake monkey.

In this paper, we show that a significantly larger number of metabolites can be detected by $^1$H MRS in vivo in the anesthetized monkey; quantification included prior knowledge from simulated metabolites and MMs. In addition, we present the first data from single-voxel $^1$H MRS in the awake monkey using a 7-T MR system with a vertical bore. Preliminary results have been presented in abstract form [31,34].

MRS in the alert monkey presents technical challenges. Typically, in physiology experiments with trained monkeys, the restraint of the animal’s head by means of a 3-point head post is sufficient for the acquisition of reliable data. In contrast, acquisition of quality MR spectra is, expectedly, sensitive to body movement. Thus, we first set out to evaluate the effects of mouth (jaw and tongue), arm, leg and rump movement on $^1$H spectral acquisition and quality. In addition, we examined the extent of well-known $B_0$ fluctuations during respiration [35–39]. Magnetic field shifts induced by gross magnetic susceptibility changes were indeed previously modeled by a $1/r^3$ function, whereby $r$ is the distance from the chest in the main direction of the magnetic field [35]. Based on this model, dynamic frequency shifts as well as shim changes (mostly components in the z-direction) induced by respiration were expected for single-voxel MRS.

2. Methods

2.1. MR system

A novel vertical 7 T/60 cm MR system (Bruker BioSpin, Ettlingen, Germany) was set up, in which multimodal MR imaging, MR spectroscopy and simultaneous electrophysiology can be performed in the anesthetized and the awake behaving monkey. Details of this system were described recently [40]. Briefly, the system is equipped with a gradient coil of 38-cm inner diameter that achieves a maximum of 80 mT/m gradient strength per channel in less than 200 μs. Passive sound insulation inside the gradient coil (33-cm inner diameter) provides a 30-dB noise reduction. The system is equipped with three radiofrequency (rf) transmission channels for all nuclei of interest, from which two can be used for dual $^1$H transmission. Four receiving channels are available for parallel imaging and simultaneous multinuclear spectroscopy.

For $^1$H experiments in the anesthetized monkey, a combination of rf coils at 300 MHz was used with separate transmission-only and receive-only (TORO) coils, as described previously [40,41]. It consisted of a 125-mm-diameter actively decoupled saddle coil for whole head excitation and a 30-mm receive-only surface coil intended for high signal-to-noise ratio (SNR) in studies with a localized area-of-interest. A 60-mm surface coil in transceiver mode [40] was used for the experiments in the awake monkey.

2.2. Subjects and transport system

For all experiments, rhesus monkeys (Macaca mulatta) with body weights of 4–5 kg were used. All procedures and experiments were approved by the local authorities (Regierungspräsidium) and were in full compliance with the guidelines of the European Community (EUVD 86/609/ECC) for the care and use of laboratory animals. Surgical procedures, anesthesia and details of the animal setup were described recently [41].

A prototype chair for awake primates was custom designed and built to accommodate rewards for the animal and motion sensors for the monitoring of body and jaw movement, visual stimulus presentation and positioning of the

<p>| Table 1 Spectral components derived from MMs as used in the LCModel analysis |
|--------------------------|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Label</th>
<th>MM position (ppm)</th>
<th>MM width (ppm)</th>
<th>Intensity in rat (a.u.)</th>
<th>Intensity in monkey (a.u.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>0.88</td>
<td>0.10</td>
<td>0.97</td>
<td>0.70 (−28%)</td>
</tr>
<tr>
<td>M9a</td>
<td>0.94</td>
<td>0.10</td>
<td>1.03</td>
<td>1.74 (+69%)</td>
</tr>
<tr>
<td>M2</td>
<td>1.22</td>
<td>0.10</td>
<td>0.58</td>
<td>0.58</td>
</tr>
<tr>
<td>M12</td>
<td>1.41</td>
<td>0.10</td>
<td>1.19</td>
<td>1.58 (+33%)</td>
</tr>
<tr>
<td>M3</td>
<td>1.70</td>
<td>0.15</td>
<td>1.02</td>
<td>1.19 (+17%)</td>
</tr>
<tr>
<td>M14</td>
<td>2.05</td>
<td>0.25</td>
<td>3.65</td>
<td>2.24 (−39%)</td>
</tr>
<tr>
<td>M5</td>
<td>2.29</td>
<td>0.15</td>
<td>1.02</td>
<td>1.37 (+34%)</td>
</tr>
<tr>
<td>M17</td>
<td>2.54</td>
<td>0.25</td>
<td>0.82</td>
<td>0 (−100%)</td>
</tr>
<tr>
<td>M21</td>
<td>2.74</td>
<td>0.10</td>
<td>0.19</td>
<td>0 (−100%)</td>
</tr>
<tr>
<td>M25</td>
<td>3.00</td>
<td>0.10</td>
<td>0.79</td>
<td>0.79</td>
</tr>
<tr>
<td>M30</td>
<td>3.22</td>
<td>0.10</td>
<td>0.65</td>
<td>0.65</td>
</tr>
<tr>
<td>M32</td>
<td>3.80</td>
<td>0.35</td>
<td>2.96</td>
<td>2.96</td>
</tr>
<tr>
<td>M38</td>
<td>4.30</td>
<td>0.35</td>
<td>0.77</td>
<td>0.77</td>
</tr>
</tbody>
</table>

* Peak positions were taken from data in rat brain, which were deduced from dialyzed cytosolic fractions [12,13] and in vivo metabolite-nulled $^1$H MRS spectra [7].

* The $^1$H spectrum from monkey brain (see Fig. 1) was fitted to adjust MM peak widths and relative intensities.

* The $^1$H spectrum from monkey brain (Fig. 1) was fitted to adjust the relative intensities of the MM, keeping position and width fixed. M09, M14, M17 and M23 intensities were increased, whereas M21, M25 and M27 intensities were decreased compared to rat data.

* Alternative labeling according to the nomenclature of Behar and Ogino [13], which marks only major peaks.
the electrophysiology apparatus, when necessary. The chair was inserted into the magnet with a vertical transport system using 2-m spindle drives and magnetically screened motors with a software-controlled gear and an emergency remove feature. All cables connecting the proximal end of the recording and monitoring devices to the main equipment were fed through a filter panel at the lower end of the chair. A similar chair was constructed for the experiments on anesthetized animals [40].

2.3. Data acquisition: $^1$H single-voxel spectroscopy

A single-voxel STEAM sequence was used for localized $^1$H MR spectroscopy. Short echo times of 4–10 ms were used to minimize the signal attenuation due to transverse relaxation ($T_2$) and to maximize signals of J-coupled spin systems. “Sinc” rf pulses were used for localized excitation. Water was suppressed with a “variable pulse power and optimized relaxation delays” module (VAPOR) using seven pulses and delays of typically 150, 100, 100, 90, 90, 55 and 25 ms [9]. The water suppression band width was ±70 and ±150 Hz at the 1% and 99% level, respectively.

Since the shim voxel was oriented in the main coordinate axes $x$, $y$, $z$ (nonoblique), the monkey’s head was rotated by 30–45° to achieve optimal shimming for voxels confined within the cortex of the operculum. For localized $^1$H spectroscopy, single-voxel spectroscopies were usually selected approximately 10–15 mm away from the cortical surface anterior to the visual cortex of the right hemisphere.

2.4. Automated shimming using “FASTMAP Scout”

Adjustment of the three first- and five second-order shim terms was accomplished with FASTMAP [42]. A procedure for automated shim operation was developed based on a Tcl/Tk program interacting and controlling a serial MR acquisition using ParaVision software. The only interactive step was the selection of shim voxel size and position at the beginning; adjustment of rf power, receiver gain and frequency were done automatically and could be updated before each shim step.

An important point in the automation was the acquisition of a reference scan subsequent to each FASTMAP shim step. In the reference scan, a single-voxel water signal was

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Fig. 1. In vivo $^1$H NMR metabolite spectrum from a 125-µl voxel in the monkey brain acquired at 7 T with short echo time. The anatomical image (transverse slice, clipped) shows the position of the voxel. The head of the monkey was rotated by 45° in the transverse plane as indicated by the bars pointing to the anterior–posterior (A) and left–right (R) orientation. The spectrum reveals excellent water suppression and baseline definition. The line width was 10.5 Hz for water and 9 Hz for metabolites (measured on the Cr/PCr CH$_3$ peak at 3.0 ppm). The predominant resonances are Cr/PCr and NAA/NAAG as indicated; in the region from 1 to 2 ppm, several resonances from MMs are visible. Acquisition parameters (scan E02.lb1): single-voxel spectroscopy using a STEAM sequence with VAPOR water suppression, voxel size 5×5×5 mm$^3$, NA 4, SW 3 kHz, TE 10 ms, TM 10 ms, TR 4 s, NR=256. Resolution enhancement was done with exponential/Gaussian windowing (lb=−0.4 Hz, gb=0.25). Multislice spin echo sequence, field of view 5.12×5.12 cm$^2$, matrix 256×256, seven slices with 1-mm thickness, nominal resolution 200×200×1000 µm$^3$, NA 1, SW 50 kHz, TE 15 ms, 12 echoes, TR 5 s.
acquired at the center of the shim voxel, which was used to calculate the on-resonance frequency, receiver gain and optimal phase evolution time, as well as for performing a dynamic update of these parameters and the shims. The optimal phase evolution time was set to the time at which the signal had decayed by a factor \( S_0 \cdot e^{-1} \), which was correlated with the current shim quality in the localized voxel. The acquisition of FASTMAP scans with an optimal evolution time was shown to maximize the SNR in the measured phase distributions along the six projections and improve the accuracy in the FASTMAP fit [42].

The FASTMAP Scout performed a dynamic evaluation of the required shim calculations (i.e., whether the calculations should involve first-order shims only or both first- and second-order shims), thus, optimizing the total time for shim duration and accuracy. The display of the calculated phase evolution time and shim values after each shim step allowed the operator to assess dynamically the current shim situation during the progressing measurements. At the end, the field maps along the six projections were plotted to display the final shim results. The total duration of the automated shimming was less than 2 min (using a TR of 1 s and a settling period of 10 s for Z2 shim changes). A “history” record allowed the user to reload any intermediate shim condition at a later time.

In the anesthetized monkey, the line width of the singlet \(^1\)H metabolite resonances (as measured on Cr/PCr at 3.0 ppm) was typically 9–11 Hz (0.03–0.037 ppm). In the awake monkey, line widths were broader (15–17 Hz, 0.05–0.057 ppm) as result of second-order shim current limitations of

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Fig. 2. Quantitative analysis of brain metabolites by frequency domain fitting of the data shown in Fig. 1. (A) Residual, MM (bold, solid) and spline (dotted) baseline of the fit using LCModel. (B, C) Amplified MM baseline and individual fitted peaks labeled M09 through M38, as fitted to the \(^1\)H metabolite spectrum in the monkey brain in vivo. For comparison, an MM spectrum from rat brain (dotted curve) [7] is shown from which MM widths were deduced (for details, see Table 1). The whole MM spectrum as the sum of all components was used as a “simulated” spectrum in the basis set utilizing prior knowledge. This approach, using a single MM spectrum instead of fitting 12 MM components individually, reduced the variances and bias for the metabolites of interest. The spline baseline [dotted curve in (A)] was nearly flat. The full results from the LCModel analysis are reported in Table 2 showing a neurochemical profile of 16 metabolites in the monkey brain. Parameters (scan E02.lb1): see Fig. 1; here, no line broadening or resolution enhancement was done.
the hardware originating in a different head holder and setup in the vicinity of the head. It was observed in some scans of awake monkeys that shim performance of FASTMAP was degraded by motion, in which case, the shim procedure was repeated.

2.5. Data processing

The data conversion and analysis was done with custom MATLAB-based software (MATLAB 6.5; The Mathworks, Natick, USA); it performed the automated phase and frequency correction as well as the determination of spectral parameters such as integral, amplitude, line width, position (frequency) and phase from dynamic series of water or metabolite spectra. In vivo metabolite spectra were referenced to the methyl signal of NAA (δ=2.009 ppm).

2.5.1. Preprocessing

After reading of FID data, conversion from digital to analog format was performed similar to the Bruker routine convda, which was basically a circular left shift operation of an appropriate number of points. Eddy-current correction was applied to the metabolite spectra to rewind the phase evolution of the FID as calculated from a reference water signal.

2.5.2. Determination of peak integral, amplitude and line width of the water signal

Peak integral was determined from the magnitude of the averaged 20 initial points of the time domain data. Peak amplitude and line width were determined in the frequency (spectral) domain after moderate exponential apodization, FT and magnitude calculation. The line width was determined as the full width at half maximum (FWHM). To determine the accurate peak amplitude and line width, spline interpolation was used.

2.5.3. Determination of peak position (frequency)

Frequency changes in dynamic series were determined relative to a reference spectrum (typically the first one in a series). Two different methods were used either in the time or the frequency (spectral) domain:

1) In the time domain, the change of phase with time was calculated relative to the reference FID. The frequency change was determined directly from the slope of a linear regression of the relative phase over time. This procedure was very fast and worked reliably for spectral series with good SNR, like the water signal series. However, for single-shot metabolite spectral series with small SNR, it was not robust against noise.

2) In the spectral domain, the change in the frequency is reflected by a change in the peak position. Exponential apodization of 10–20 Hz was applied before FT. In the magnitude spectrum, a peak was selected, either the residual water signal or the NAA resonance at 2.0 ppm. Thereafter, the position of the peak maximum was determined by spline interpolation around the maximum intensity. This procedure worked well and was robust even for noisy data, since SNR was enhanced by the exponential apodization.

2.5.4. Frequency correction

Before averaging, the MRS data were processed by multiplication of the FID with an inverse linear phase derived from the relative frequency changes. This could account for the significant effects of frequency variations causing line broadening.

2.6. Metabolite quantification

Quantification was based on frequency domain analysis using “Linear Combination of Model spectra” (LCModel, version 6.0.1) [43,44], as described previously [7]. In vivo spectra were fitted by a linear combination of a set of basis spectra by means of a constrained regularization algorithm minimizing the difference between this combination and line shape and baseline obtained by the data. The method used the spectral pattern of each basis spectrum without further analysis.

Basis spectra were simulated using NMR-SIM 3.2 (Bruker BioSpin) as described recently [45], based on chemical shifts and coupling constants reported in the literature [46]. The following 18 metabolites were included in the analysis: alanine (Ala), aspartate (Asp), glycerophosphorylcholine (GPC), phosphorylcholine (PC), creatine (Cr), phosphocreatine (PCr), γ-aminobutyric acid (GABA), glutamic acid (Glu), glutamine (Gln), glutamine plus glutamate (Glu+Gln), creatine plus phosphocreatine (Cr+PCr), myo-inositol (Ins), taurine (Tau), N-acetylaspartate (NAA), N-acetylaspartylglutamate (NAAG), N-acetylaspartylglutamate plus N-acetylaspartate (NAA+NAAG), N-acetylaspartic lactate, and scyllo-inositol (Scyllo). The following 18 metabolites were included in the analysis: alanine (Ala), aspartate (Asp), glycerophosphorylcholine (GPC), phosphorylcholine (PC), creatine (Cr), phosphocreatine (PCr), γ-aminobutyric acid (GABA), glutamic acid (Glu), glutamine (Gln), glutamine plus glutamate (Glu+Gln), creatine plus phosphocreatine (Cr+PCr), myo-inositol (Ins), taurine (Tau), N-acetylaspartate (NAA), N-acetylaspartylglutamate (NAAG), N-acetylaspartylglutamate plus N-acetylaspartate (NAA+NAAG), N-acetylaspartic lactate, and scyllo-inositol (Scyllo). The following 18 metabolites were included in the analysis: alanine (Ala), aspartate (Asp), glycerophosphorylcholine (GPC), phosphorylcholine (PC), creatine (Cr), phosphocreatine (PCr), γ-aminobutyric acid (GABA), glutamic acid (Glu), glutamine (Gln), glutamine plus glutamate (Glu+Gln), creatine plus phosphocreatine (Cr+PCr), myo-inositol (Ins), taurine (Tau), N-acetylaspartate (NAA), N-acetylaspartylglutamate (NAAG), N-acetylaspartylglutamate plus N-acetylaspartate (NAA+NAAG), N-acetylaspartic lactate, and scyllo-inositol (Scyllo). The following 18 metabolites were included in the analysis: alanine (Ala), aspartate (Asp), glycerophosphorylcholine (GPC), phosphorylcholine (PC), creatine (Cr), phosphocreatine (PCr), γ-aminobutyric acid (GABA), glutamic acid (Glu), glutamine (Gln), glutamine plus glutamate (Glu+Gln), creatine plus phosphocreatine (Cr+PCr), myo-inositol (Ins), taurine (Tau), N-acetylaspartate (NAA), N-acetylaspartylglutamate (NAAG), N-acetylaspartylglutamate plus N-acetylaspartate (NAA+NAAG), N-acetylaspartic lactate, and scyllo-inositol (Scyllo). The following 18 metabolites were included in the analysis: alanine (Ala), aspartate (Asp), glycerophosphorylcholine (GPC), phosphorylcholine (PC), creatine (Cr), phosphocreatine (PCr), γ-aminobutyric acid (GABA), glutamic acid (Glu), glutamine (Gln), glutamine plus glutamate (Glu+Gln), creatine plus phosphocreatine (Cr+PCr), myo-inositol (Ins), taurine (Tau), N-acetylaspartate (NAA), N-acetylaspartylglutamate (NAAG), N-acetylaspartylglutamate plus N-acetylaspartate (NAA+NAAG), N-acetylaspartic lactate, and scyllo-inositol (Scyllo). The following 18 metabolites were included in the analysis: alanine (Ala), aspartate (Asp), glycerophosphorylcholine (GPC), phosphorylcholine (PC), creatine (Cr), phosphocreatine (PCr), γ-aminobutyric acid (GABA), glutamic acid (Glu), glutamine (Gln), glutamine plus glutamate (Glu+Gln), creatine plus phosphocreatine (Cr+PCr), myo-inositol (Ins), taurine (Tau), N-acetylaspartate (NAA), N-acetylaspartylglutamate (NAAG), N-acetylaspartylglutamate plus N-acetylaspartate (NAA+NAAG), N-acetylaspartic lactate, and scyllo-inositol (Scyllo). The following 18 metabolites were included in the analysis: alanine (Ala), aspartate (Asp), glycerophosphorylcholine (GPC), phosphorylcholine (PC), creatine (Cr), phosphocreatine (PCr), γ-aminobutyric acid (GABA), glutamic acid (Glu), glutamine (Gln), glutamine plus glutamate (Glu+Gln), creatine plus phosphocreatine (Cr+PCr), myo-inositol (Ins), taurine (Tau), N-acetylaspartate (NAA), N-acetylaspartylglutamate (NAAG), N-acetylaspartylglutamate plus N-acetylaspartate (NAA+NAAG), N-acetylaspartic lactate, and scyllo-inositol (Scyllo).
cose (Glc), glutamine (Gln), glutamate (Glu), glutathione (GSH), myo-inositol (Ins), scyllo-inositol (Scyllo), lactate (Lac), N-acetylaspartate (NAA), N-acetylaspartylglutamate (NAAG), phosphorylethanolamine (PE) and taurine (Tau). After an initial LCModel analysis, metabolites with Cramér-Rao lower bounds (CR) >50% were omitted to reduce the variance, which was the case for Lac, for example.

Using a new feature of the LCModel software, a single simulated MM spectrum was included in the analysis, which consisted of 12 MM peaks and was calculated during

Fig. 3. In vivo ¹H metabolite spectrum from the monkey brain at 7 T (top) and fitted model spectra using frequency domain analysis with LCModel. All spectra are consistently scaled, the major resonances of Cr/PCr (3.03 ppm) and NAA/NAAG (2.01 ppm) are cut as indicated (~). Sixteen metabolites were found in vivo; Lac was below detection limit. With narrow metabolite line widths, even the Cr and PCr resonance could be quantified separately with an error of 6% and 7%, respectively. myo-Inositol (Ins) and N-acetylaspartate (NAA) show nicely resolved J-coupling patterns; also, Scyllo and NAAG (dotted curve) were detected. Most importantly, the γCH₂ (C4) resonance of Gln (dotted) could be separated from γCH₂ of Glu, which is an advantage of increased spectral dispersion at higher magnetic field. Low concentration metabolites like Ala, Asp, Cho, GABA, Glc, GSH, PE and Tau were found. The full quantitative analysis is given in Table 2.
run time. Details of MM peak positions, line widths and intensities are reported in Table 1; the rationale of the procedure is described below. In the LCModel fit, only a zero-order phase correction was performed resulting from proper timing of the first sampled data point in the pulse sequence. Metabolite concentrations were absolutely scaled assuming 8-mM total Cr in brain tissue [47].

3. Results and discussion

3.1. Single-voxel $^1$H spectroscopy in the anesthetized monkey

An in vivo $^1$H metabolite spectrum from a 125-μl voxel (5-mm cube), consisting to a large extent of the visual cortex of the animal, is shown in Fig. 1. The insert indicates the position of the voxel plotted onto a transverse anatomical image, which was placed well in the brain at about a 4-mm distance from the cortical surface (the center of the square is approximately 7 mm from the pial surface). The $^1$H spectrum obtained was characterized by good water suppression (see residual water peak at 4.6 ppm in Fig. 1) and excellent baseline definition (only zero-order phase corrected). The dominating resonances at 2 and 3 ppm were assigned to NAA/NAAG and Cr/PCr based on their peak position. In the region of 0.5–2 ppm, several in-phase resonances were observed attributed to MMs, which were detectable due to the relatively short echo time of 10 ms. The line width was 0.030 ppm (9 Hz) in the metabolite spectrum as measured on the Cr/PCr CH$_3$ peak at 3.0 ppm; the line width was 0.035 ppm (10.5 Hz) for the water signal in the same voxel. The questions tackled in this study could be addressed by acquiring data from one hemisphere only. The head of the monkey was therefore rotated by 30–45° in the transverse plane to achieve optimal shimming. Signal-to-noise ratio was optimized by a coil-assembly with a saddle coil for excitation and a small diameter (30 mm) surface coil for reception. As expected, better shimming was possible for voxels with a few millimeters distance from the cortical surface.

3.2. Quantification of $^1$H metabolite spectra using simulated MM resonances

Quantitative analysis of brain metabolites was done with curve fitting in the frequency domain using LCModel. In addition to simulated metabolite spectra, we used a simulated MM spectrum over the whole spectral region from 0 to 4.2 ppm. The result from the LCModel fit is shown in Fig. 2A with the residuals of the fit (top) and the fitted MM and spline baseline (solid and dotted line). With the use of the simulated MM baseline, the residual spline baseline in the fit was nearly flat. The individual MM components (M09–M38) and their summed contribution can be seen in Fig. 2B and C (intensity amplified). Prior knowledge was incorporated by using peak positions from Refs. [12] and [13] and MM line widths from an MM spectrum in rat [7] (dotted curve in Fig. 2B).

A summary of the MM data used for the simulated MM spectrum is given in Table 1. The fitted MM line widths were between 0.1 and 0.35 ppm (column 2). Comparing the fitted MM peak intensities between rat and monkey data (column 3 and 4) shows that many MM peaks were very similar in intensity (M02, M30, M32, M38), while others needed moderate adjustment of 20–40% (M09, M14, M17, M21, M23). Two peaks (M25 and M27) were absent in the monkey.

It has been shown that there is only a minor difference between two fitting approaches using either the LCModel built-in spline baseline alone or together with an experimentally measured MM spectrum [7]. This is because the LCModel spline baseline can account for most of the underlying MM resonances and other baseline signals, for example, residual water, lipids or outer-volume fat contamination. Using the experimentally measured MM demonstrated that it was indeed the case that most of the underlying resonances at short echo time could be explained by MM contributions, since the remaining spline baseline was found to be flat [7]. However, the inclusion of a single MM baseline

![Fig. 4. Respiration-induced B0 fluctuations in the anesthetized (A) and the awake (B) monkey brain as detected by single-voxel $^1$H MRS of the water signal. During the 2-min scans, the frequency change in the anesthetized monkey (A) was less than 1 Hz p-p and no drift was observed. In the awake monkey (B), frequency changes were approximately doubled (2 Hz p-p) and significant drifts were observed. This was due to the fact that frequency changes in the awake monkey were not only caused by breathing. Movement of body parts like arms, legs or changes of the seat position (rump) additionally caused frequency changes that ranged from small to very large, depending on the type of body movement. Acquisition parameters (scans G02, gM1, B02, gN1): single-voxel localized spectroscopy (water signal) using a STEAM sequence, voxel size 10×10×10/5×5×5 mm$^3$, SW 3 kHz, TE 5 ms, TM 10 ms, TR 500 ms, NR 256.](image-url)
reduced the variances for fitted metabolites such as GABA, GSH, Glc, especially with low concentrations; therefore, the fitted concentrations were somewhat reduced by imposing the stronger constraint on the baseline. Similarly, we found that an inclusion of 12 simulated multiple MM resonances (M09–M38) increased the variance and biased the global LCModel fit, specifically for low concentration metabolites (data not shown). Therefore, in the present study, we chose to use the above approach with a single MM baseline in the fit (containing a weighted sum of 12 peaks) with prior knowledge about peak position and width, and empirically determined amplitudes.

The quantitative results from the LCModel analysis of $^1$H brain metabolite signals are reported in Table 2 and Fig. 3, showing a neurochemical profile of 16 metabolites in the monkey brain. The reported concentrations were scaled relative to total Cr, which was assumed to be 8 mM [47]. Major metabolites with higher concentrations (>3 mM) were Glu, NAA, Cr, PCr and Ins. Notably, similar metabolites, which are similar in their resonances, could be reliably separated: Gln from Glu, NAAG from NAA, Cr and PCr. This demonstrates the excellent spectral features, narrow line widths and good sensitivity of the $^1$H spectrum at high field. Lower concentration metabolites (<3 mM)

Fig. 5. Respiration/body motion–induced effects in the awake monkey brain as detected by single-voxel $^1$H MRS. Dynamic changes in the water resonance can be seen in the amplitude (A), the line width (B) and the peak position/frequency change (C). In the first 1.5 min of the scan (period I), only respiration-induced changes were observed mainly in the frequency (C). During the second part of the scan (periods II and III), four high magnitude events (motion periods IIa–d) occurred, as indicated by the vertical dotted lines. These could be attributed to body motion and caused significant changes in all spectral parameters, that is, peak amplitude, width and position; amplitude drops up to 50% were most significant. In the periods between motion periods (rest periods IIIa–c), the monkey kept still and only small frequency modulations due to respiration were observed. In periods III, the monkey most likely assumed a new seat position compared to period I, because the level of frequency changed from 0 to 7 Hz as indicated by the horizontal dotted lines. Interestingly, only frequency (C) changed between period I and III, whereas neither amplitude and line width were altered. Acquisition parameters (C00.mI1): single-voxel localized spectroscopy (water signal) using a STEAM sequence, voxel size $5 \times 5 \times 5$ mm$^3$, NA 1, SW 3 kHz, TE 5 ms, TM 10 ms, TR 250 ms, NR 1024. The initial 1.5-min period of the 4.5-min scan are not shown because it was similar to the rest period I.
were Ala, Asp, Cho, GABA, Glc, GSH, PE and Tau. Lactate (at 1.32/4.11 ppm) was below the detection limit (CR > 50%).

The reliability of the major metabolites, as estimated in the CR from the LCModel fit, was very good, typically 1–3%. Cramér-Rao lower bounds of most other metabolites were in the order of 10%, except NAA (19%), Scylo (16%), Ala (22%) and Tau (30%). Metabolite ratios were found to be 1.39 for [NAA+NAAG]/[Cr+PCr], 1.38 for [Glu+Gln]/[Cr+PCr], 0.09 for [Cho]/[Cr+PCr] and 1.16 for [Cr]/[PCr], which are similar to those reported in the literature ([7] and refs. therein). [Tau] was very low, which was, however, not surprising, because large variations of this metabolite in different brain areas and different species are known to exist. We note here in passing that the absence of detectable Lac is additional, albeit serendipitous, evidence for optimal anesthesia maintenance in our experiments.

The reported metabolite concentrations in monkey are similar to those estimated from 1H MRS data in humans at 7 T [47], wherein metabolite ratios were reported to be 1.31 for [NAA+NAAG]/[Cr+PCr], 1.38 for [Glu+Gln]/[Cr+PCr] and 0.11 for [Cho]/[Cr+PCr]. Similarly, the reported concentrations of Tau with 1.5 mM (CR 8%) and Lac with 0.37 mM (CR 17%) were very low in the human spectroscopy data [47].

In Fig. 3, the fitted model spectra are plotted with consistent scaling together with the in vivo 1H spectrum (top). The major resonances of Cr/PCr (3.03 ppm) and NAA/NAAG (2.01 ppm) were cut as indicated (–). The fitted CH2 resonances of Cr and PCr were separated at 3.9 ppm as indicated by a broader in vivo line width compared to the CH3 resonance at 3.0 ppm; thus, both [Cr] and [PCr] had an error below 10%. myo-Inositol (Ins) and N-acetylaspartate (NAA) showed nicely resolved J-coupling patterns. scyllo-Inositol (Scylo) and N-acetylaspartylglutamate (NAAG) (dotted curves) could be separated from the major metabolites. Most importantly, the γCH2 (C4) resonances of Gln and Glu (dotted and solid curve) could be resolved, which is a prerequisite for tracking glutamatergic pathways. Additional metabolites with low concentrations (<3 mM) are also shown, including Ala, Asp, Cho, GABA, Glc, GSH, PE and Tau.

3.3. B0 fluctuations in monkey MRS

Single-voxel 1H spectroscopy was performed also in the awake monkey. Because only the head of the monkey was constrained by a 3-point head holder, commonly used in our laboratory, the effect of movement of body parts (arms, legs, rump) and mouth (jaw, tongue) on 1H spectra had to be evaluated and correction strategies needed to be developed.

Firstly, the influence of respiration-induced B0 fluctuations in the anesthetized (A) and the awake (B) monkey. Frequency was calculated from the localized water signal from a 10-mm cube (1H MRS) acquired with a TR of 0.5 s. During the 2-min scans, the frequency change in the anesthetized monkey (Fig. 4A) was less than 1 Hz p-p and no drift was observed. The frequency changes in the awake monkey (Fig. 4B) were approximately doubled (2 Hz p-p) and significant drifts were observed. These additional drifts could be induced by body motion.

Inspection of the data showed that movements of the arms and/or legs and changes of the seat position (rump-motion) frequently did cause small but occasionally catastrophic frequency changes depending on the type and the extent of body movement. The change of the baseline therefore could be attributed to small movements of the monkey inside the chair, for example, arm or leg, which caused susceptibility-induced B0 fluctuations at the position of the head similar to those caused by respiration.

The effect of very large body movements of the awake monkey on MRS data is shown in Fig. 5. A dynamic single-voxel 1H MRS series of the water signal was recorded with a TR of 250 ms. The spectral parameters relative peak amplitude, line width and relative peak position (frequency change) were extracted to characterize the dynamic changes. Different periods were observed and indicated by the labeled bars in Fig. 5: resting period (I), body motion periods (IIa–d) and resting periods in-between body motions (IIIa–c). In the first 1.5 min of the scan (resting...
period I), mainly respiration-induced changes in frequency were observed, whereas amplitude and line width remained unaltered. The frequency baseline revealed a small drift in the order of an additional 1–2 Hz. In the second part of the scan, four “high magnitude events” attributed to body motion occurred as indicated by the vertical dotted lines in Fig. 5 (body motion periods Ila–d). The relative peak amplitude dropped between 10% and 50%, the line width changed by 4 Hz p-p (±10%) and the peak position varied by more than 40 Hz p-p. Changes in the peak integral were also calculated, which, however, revealed very similar dynamics to those of the peak amplitude (data not shown). In the resting periods between body motions (IIa–c), again, only respiration-induced 2-Hz changes in the frequency and no alterations in amplitude and line width were observed. Interestingly, the frequency baseline changed to a new level at 7 Hz as indicated by the horizontal dotted lines. This observation is most likely due to the fact that the animal took up a new seat position in periods IIa–c compared to period I, but subsequently remained still for that period.

The line width of a single spectrum (at the beginning of the series) was 15.6 Hz, which increased to 19.1 Hz for the averaged spectrum, when considering all spectra from periods I, II and III together, including the body motion periods. In comparison, when a frequency correction was applied before averaging, the line width of the averaged spectrum was 15.8 Hz, close to the one of a single-shot spectrum (15.6 Hz). However, strong amplitude variations were still apparent and not corrected. The line width of the final averaged spectrum excluding the body motion periods (IIa–d) was 15.8 Hz, and no amplitude variations were apparent.

These observations led us to the following acquisition and correction strategy for 1H MRS in the awake monkey: (1) No averaging was performed at the acquisition step, and each repetition was stored separately to allow flexibility in postprocessing. (2) The water suppression was not adjusted perfectly to improve SNR for the frequency correction. (3) The temporal MRS series was tracked for major artifacts due to body movement based on significant changes in peak amplitude and frequency. Periods with major body motion were then discarded from the analysis. (4) Finally, a frequency correction was performed based on the tracked dynamic frequency changes of the residual water signal. Spectra were then averaged and further processed.

This strategy was mainly based on the observations that major body motion could be easily detected by significant dynamic changes in amplitude and frequency. Furthermore, subsequent resting periods, even with major body motion between them, did not show significant changes in the line width. As constant frequency and narrow line width are both critically important for high spectral quality (while amplitude mainly contributes to the SNR), a two-step approach was sufficient to obtain optimal results in the averaged spectra: rejection of periods with major body motion and frequency correction before averaging.

Dynamic $B_0$ changes of about 2 Hz p-p due to respiration were very similar to those reported previously for the human brain at 7 T [39]. Significant changes in the spectral line width and amplitude were observed during major body motion attributed to spatially dependent magnetic field changes (“shim”). Small frequency changes, for example, the drifting frequency baseline or periods before/after motion, were related to small body motions and did not show a significant spatial $B_0$ dependence and shim effect in the order of the voxel size of 10 mm and smaller.

### 3.4. 1H MR spectroscopy in the awake monkey

A typical in vivo 1H metabolite spectrum from the awake monkey brain is shown in Fig. 6, acquired from a 1-ml

### Table 3

<table>
<thead>
<tr>
<th>Study*</th>
<th>Concentration (mM) [CR (%)]</th>
<th>Mean±S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C00.mN1-17</td>
<td>C00.mO1-17</td>
</tr>
<tr>
<td>NAA+NAAG</td>
<td>10.4 (4)</td>
<td>11.2 (3)</td>
</tr>
<tr>
<td>Gln</td>
<td>–</td>
<td>1.8 (20)</td>
</tr>
<tr>
<td>Glu</td>
<td>–</td>
<td>7.1 (6)</td>
</tr>
<tr>
<td>Glu+Gln</td>
<td>8.7 (9)</td>
<td>8.9 (6)</td>
</tr>
<tr>
<td>Cr</td>
<td>–</td>
<td>3.8 (20)</td>
</tr>
<tr>
<td>PCr</td>
<td>–</td>
<td>4.2 (17)</td>
</tr>
<tr>
<td>Cr+PCr</td>
<td>8.0 (5)</td>
<td>8.0 (4)</td>
</tr>
<tr>
<td>Ins</td>
<td>5.9 (8)</td>
<td>5.9 (6)</td>
</tr>
<tr>
<td>Cho (GPC+PC)</td>
<td>1.2 (11)</td>
<td>0.86 (14)</td>
</tr>
<tr>
<td>GABA</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>GSH</td>
<td>2.3 (14)</td>
<td>1.4 (11)</td>
</tr>
<tr>
<td>PE</td>
<td>–</td>
<td>1.7 (24)</td>
</tr>
<tr>
<td>NR</td>
<td>320/512</td>
<td>330/512</td>
</tr>
<tr>
<td>TE (ms)</td>
<td>4</td>
<td>10</td>
</tr>
</tbody>
</table>
voxel with short echo time of 4–10 ms. Good water suppression and baseline definition, but only a medium-quality line width, were achieved compared to the 1H spectrum from anesthetized monkeys in Fig. 1. Data from five studies with quantitative analysis of metabolite concentrations are listed in Table 3.

An increased line width in the awake monkey studies was already observed in the single-shot water reference spectra and was therefore not due to possible motion artifacts and subsequent peak broadening, but resulted from shim hardware limitations. Due to a different setup and head posts, some shim currents reached maximum values and were below the desired value calculated by FASTMAP. The observed line widths were between 15 and 17 Hz in the awake MRS studies compared to 9–11 Hz in the anesthetized monkey. In the spectrum of the awake monkeys (Fig. 6), the detailed coupling patterns of Ins and NAA, for example, were lost, unlike the spectrum from anesthetized monkeys (Fig. 1). The major consequence of a broader line width was a loss in sensitivity, particularly affecting estimates of lower concentration metabolites. This finally led to a decreased number of reliably detected metabolites.

The data from different MRS studies in the awake monkey (Table 3) showed that NAA+NAAG, Glu+Gln, Cr+PCr, Ins, Cho and GSH could be detected (CR < 30%). Mean metabolite concentrations averaged over all studies are given in the last column. The error of the major concentration metabolites was well below 10% (NAA+NAAG, Glu+Gln, Cr+PCr, Ins). In some studies, it was possible to detect Gln separately from Glu and PCr separately from Cr. The metabolite ratios were found to be 1.3 for [NAA+NAAG]/[Cr+PCr], 1.1 for [Glu+Gln]/[Cr+PCr], 0.11 for [Cho]/[Cr+PCr] and 1.4 for [Cr]/[PCr], which were similar to the data observed in Table 2, taking into account the increased error in the alert monkey data.

In the data of monkey “C00” (Table 3, column 1 and 2), nearly one third of the scans were discarded due to body motion, compared to “B02” (Table 3, columns 3 and 4), from which all scans were used. For one study, the quantification of a spectrum with only eight averages (0.5 min scan) is shown (Table 3, column 5) to demonstrate that NAA+NAAG, Glu+Gln, Cr+PCr and Ins were still detected with an error of about 10%, but Cho and GSH with an error of about 30%.

In summary, in the present paper, we demonstrate that 16 metabolites from localized 1H MR spectra can be quantified in the anesthetized monkey due to the increased sensitivity at 7 T, resulting in high SNR and spectral dispersion, and as a result of optimal shimming. For metabolite quantification, frequency-domain fitting with 18 simulated metabolite spectra and a simulated MM baseline consisting of 12 components was used. This approach and data given in Table 1 will also be useful for quantification of localized 1H MRS in other animal models, like rat or mouse, as well as in the human brain.

B0 fluctuations due to respiration and motion of body parts were evaluated for MRS in the awake monkey. Severe body motion resulted in changes to spectral parameters such as amplitude, line width and frequency with straightforward detectability. We found that in different resting periods, spectral amplitude and line width, that is, the voxel shim, were not influenced. A simple correction scheme was developed, consisting of (1) the rejection of periods with major body motion and (2) a frequency/phase correction of the remaining single spectra before averaging. In this manner, reliable detection of 6–10 brain metabolites in the awake monkey was finally made possible.

Acknowledgments

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References


