

Chapter 2

Methodology of MRS in Animal Models: Technical Challenges and Solutions

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Abstract In vivo ^1H MR spectroscopy is a unique technique, which is capable of providing neurochemical information from a selected volume of tissue noninvasively. However, the richness and reliability of neurochemical information gained by MRS depends heavily on the data acquisition and processing techniques utilized. What makes the use of MRS in neuroscience and medical research even more challenging is the fact that the most advanced MRS techniques developed in the last 15 years are not routinely provided by MR scanner vendors. This chapter provides an overview of the MRS methodology for studying animal models of human neurodegenerative diseases. The chapter's subsections focus on MRS data acquisition, processing, and metabolite quantification. The data acquisition section outlines some basic hardware requirements, B_0 shimming, water suppression, and localization techniques. The data processing section describes methods applied on acquired MRS data before metabolite quantification, such as frequency, phase, and eddy current correction. The quantification section focuses specifically on LCModel analysis. Finally, some examples, demonstrating the potentials of high-field ^1H MRS for neurochemical profiling in mice, are presented.

Keywords Localization • PRESS • STEAM • LASER • SPECIAL • B_0 shimming • FASTMAP • Chemical shift displacement error • Water suppression • Eddy currents • Quantification

Introduction

Animal models of human neurodegenerative diseases are fundamental for pre-clinical studies. In addition, enormous progress has been made in the last decade in developing new mouse models of neurodegenerative diseases by novel genetic engineering tools. An examination of these models requires advanced experimental techniques that provide accurate information about underlying

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neuropathological processes and that can be used for monitoring disease progression and evaluating the effectiveness of novel therapeutic approaches to assist drug discovery and development. In vivo ^1H MR spectroscopy is a unique method because it is capable of providing neurochemical information from a selected volume of brain tissue noninvasively [1]. This feature makes the technique ideal for longitudinal animal studies, which substantially reduces the number of used animals and avoids unnecessary animal euthanasia. Figure 2.1 shows the in vivo ^1H MR spectrum of the mouse hippocampus and demonstrates the range and type of neurochemical information achievable at 9.4 T. A reliable quantification up to 20 brain metabolites is currently feasible from the rat or mouse brain, and the metabolite detection threshold is about $0.5\text{ }\mu\text{mol/g}$. The ^1H MR spectrum in Fig. 2.1 clearly illustrates that a substantial spectral overlap is present at magnetic field strengths as high as 9.4 T. Consequently, the full scale of the neurochemical information can be obtained only if carefully optimized advanced spectroscopy acquisition and processing techniques are used. A suboptimal pulse sequence design or a modest misadjustment of acquisition parameters can substantially deteriorate the resulting spectral quality, which makes reliable quantification of metabolites impossible. As the most advanced MRS techniques and processing tools are not routinely provided by MR scanner vendors, reaching a high spectral quality and reliability of metabolite quantification can be challenging for MRS users.

The primary goal of this chapter is to review the basic principles of MRS in animal models, to improve the understanding of the challenges that MRS users might be facing, and to help potential new MRS users to choose the most appropriate approaches in order to maximize the neurochemical information that can be obtained by this technique. The chapter specifically provides an overview of the ^1H MRS methodology for studying animal models of human neurodegenerative diseases. The chapter's subsections focus on MRS data acquisition, processing, and metabolite quantification. At the end of the chapter, some examples demonstrating the potentials of high-field ^1H MRS for neurochemical profiling in mice are presented.

MRS Data Acquisition

Hardware Requirements

The hardware of modern small animal MRI scanners, in general, meets the requirements for advanced MRS. But spectroscopy has high demands especially on the performance of the second-order shim system and the design of the RF coils. Sensitivity is one of the limiting factors for MRS, because the concentration of MR detectable metabolites is by 4 orders of magnitude lower than the concentration

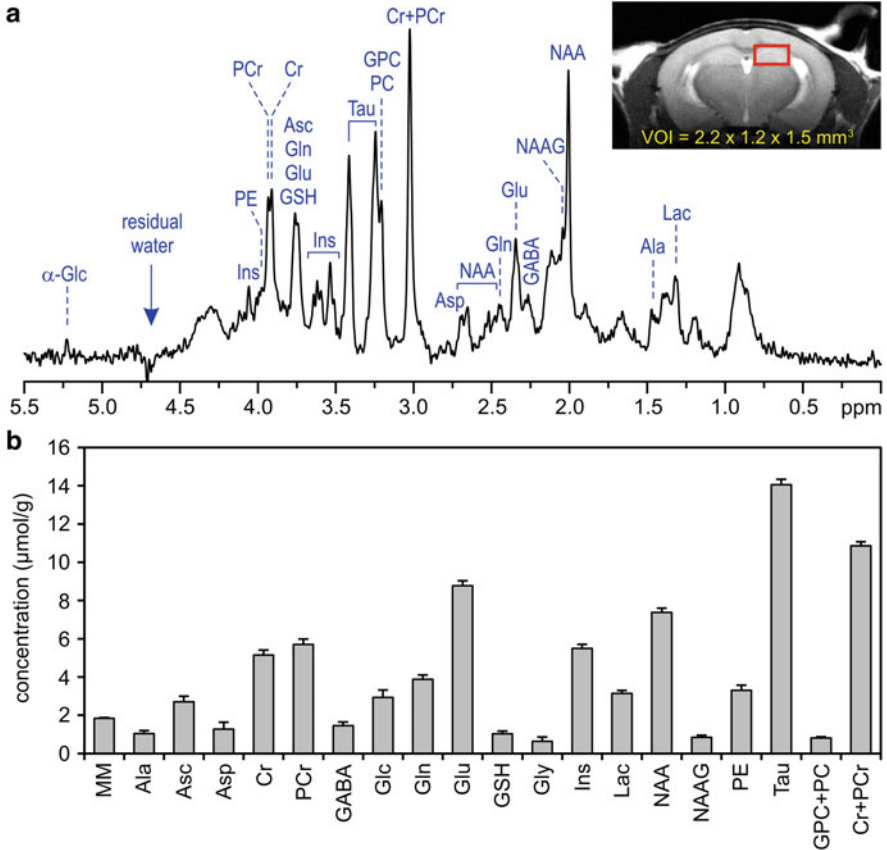


Fig. 2.1 In vivo ^1H MRS of the mouse brain at 9.4 T. (a) A representative ^1H MR spectrum acquired from the hippocampus (STEAM with OVS and VAPOR water suppression, $\text{TE} = 2$ ms, $\text{TR} = 5$ s, $\text{NT} = 320$). (b) The neurochemical profile of the hippocampus quantified from the spectrum shown in (a), LCModel, error bars indicate the estimated error of this fit (Cramér-Rao lower bounds). Abbreviations of metabolites: *MM* macromolecules, *Ala* alanine, *Asc* ascorbate, *Asp* aspartate, *Cr* creatine, *PCr* phosphocreatine, *GABA* γ -aminobutyric acid, *Glc* glucose, *Gln* glutamine, *Glu* glutamate, *GSH* glutathione, *Gly* glycine, *Ins* myo-inositol, *Lac* lactate, *NAA* *N*-acetylaspartate, *NAAG* *N*-acetylaspartylglutamate, *PE* phosphoethanolamine, *Tau* taurine, *GPC* glycerophosphocholine, *PC* phosphocholine

of water, which provides the source signal for MRI. As both the MR sensitivity and the chemical shift dispersion (in Hz) scale with the strength of the static magnetic field (B_0), ultrahigh-field MR systems (≥ 7 T) are highly beneficial for small animal MRS. Currently 9.4 T MR scanners appear to be the best compromise between cost and performance for advanced ^1H MRS of rodents.

RF Coils

Appropriate RF coils can substantially boost the sensitivity and increase the signal-to-noise ratio (SNR) of acquired data. In order to increase the sensitivity of the RF coil, the coil has to be as close as possible to the examined volume of interest (VOI) in the brain. Therefore, surface RF coils might be preferred over volume coils for brain MRS of rodents, because it is much easier to place a small surface RF coil directly on top of the head in close proximity of the VOI. An additional advantage of small surface RF coils is their spatially limited sensitivity, which significantly helps to minimize the unwanted signals arising from remote areas outside of the VOI. However, this spatially limited sensitivity may cause difficulties in acquiring MRS data from deep brain structures. One solution is to use a volume RF coil for transmission, which can generate a spatially homogeneous B_1 field, and a separate small surface RF coil only for reception. Another alternative is to use a quadrature transmit/receive surface RF coil with two geometrically decoupled single-turn loops of appropriate diameter (10–15 mm), which might be the best compromise for mouse brain MRS, providing high sensitivity and reasonable spatial homogeneity for transmit as well as for receive RF fields.

Shim System

The B_0 field homogeneity is absolutely essential for MRS, because it determines the spectral resolution. B_0 inhomogeneity over the selected VOI increases the spectral linewidth that consequently decreases the SNR and increases the overlap of metabolite resonances, which together result in compromised metabolite quantification. B_0 inhomogeneities primarily originate from susceptibility differences between air and tissue. These inhomogeneities are scaled with the strength of the B_0 field and become highly nonlinear at ultrahigh magnetic fields. Animal MR scanners are typically equipped with the hardware (shim coils and drivers) capable of generating magnetic fields of appropriate strengths and symmetries to compensate these inhomogeneities. The process of adjusting the B_0 field homogeneity is known as B_0 shimming or simply shimming. The spatial magnetic field inhomogeneities are, in general, very complex, but for small VOIs they can be sufficiently well approximated by first- and second-order shim terms.

The strength of linear shims (X, Y, Z) is not a limiting factor, because powerful gradient coils and gradient amplifiers are widely used in MR scanners for the first-order shim corrections. Therefore, the strength of the second-order shim system is critical for successful MRS at high fields. The smaller the animal head, the bigger are the field deformations, which implies that a substantially more powerful shim system is required for brain ^1H MRS in mice relative to rats. The maximum strengths of the second-order shim system recommended for the 9.4 T small animal MR scanner are at least 2000 Hz/cm^2 ($50 \text{ }\mu\text{T/cm}^2$) for XZ, YZ, and Z2 shim coils and 1000 Hz/cm^2 ($25 \text{ }\mu\text{T/cm}^2$) for XY and X2Y2 shim coils [2].

B₀ Shimming

As it was mentioned in the previous section, B_0 shimming is a key factor for MRS and reliable metabolite quantification because the separation of some metabolite resonances (signals) is only a few Hz. Successful B_0 shimming requires not only a powerful second-order shim system but also an efficient B_0 mapping technique for automatic adjustment of currents in shim coils. Methods developed for B_0 field mapping can be grouped into two categories: methods based on 3D B_0 mapping [3] and B_0 mapping along projections [4]. In both cases, B_0 mapping is based on phase evolution of the transverse magnetization in an inhomogeneous B_0 field. Small VOIs selected for ^1H MRS require high spatial resolution of the B_0 mapping method, which favors projection techniques, such as FASTMAP [4, 5] over 3D mapping techniques.

FASTMAP is a fully automatic B_0 shimming method. It maps the field along thin bars through the center of the shimmed volume. Typically, three to four iterations are necessary for very fine shimming and the entire process takes less than 2 min. The FASTMAP technique allows adjustment of B_0 homogeneity in volumes as small as 4 μL (Figs. 2.1 and 2.2a). The natural signal linewidth (assuming perfect B_0 homogeneity) is determined by the T_2 relaxation and the microscopic heterogeneity of the tissue. The linewidths of unsuppressed water signals ranging from 10 to 12 Hz are achievable at 9.4 T for most mouse brain regions. However, in some brain areas, such as the cerebellum, the spectral linewidth is broader despite perfect shimming due to intrinsic properties of the tissue [6, 7]. In general, the natural signal linewidth is smaller in neonates relative to adults due to higher water content and less myelin. Consequently, water signal linewidths as low as 8–10 Hz are achievable at 9.4 T in mouse pups [8].

Poor B_0 shimming not only increases the spectral linewidths (Fig. 2.2e) but may also significantly affect the signal lineshape. Distorted and asymmetric signal lineshapes make metabolite quantification difficult and negatively affect the reliability of assessed metabolite levels.

Localization Techniques

The basic difference between in vivo ^1H MRS and the traditional high-resolution ^1H NMR spectroscopy widely used in organic chemistry and biochemistry is the capability of MRS to acquire data from a well-defined volume of sample. Spatial localization is fundamental for in vivo applications, because MR spectra acquired from the whole animal would not provide meaningful information. Spatially selective excitation is typically based on a combination of three orthogonal slice-selective pulses (band-selective RF pulses simultaneously applied with field gradients), and the signal of interest is acquired from the intersection of these slices. In addition, localization techniques must include water suppression because the detection of brain

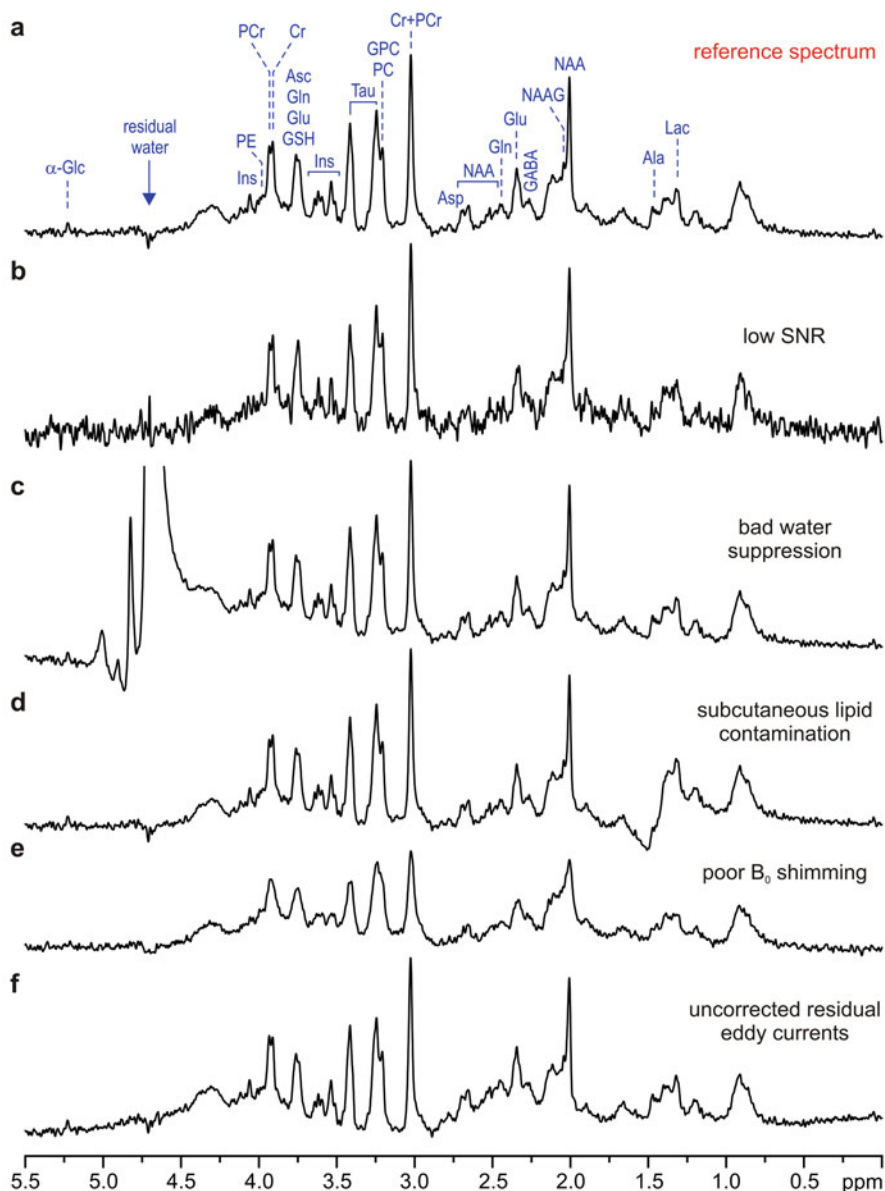


Fig. 2.2 Most important factors affecting spectral quality. (a) Reference spectrum acquired from the mouse hippocampus using STEAM localization sequence with OVS and VAPOR water suppression at 9.4 T ($TE=2$ ms, $TR=5$ s), (b) spectrum with low signal-to-noise ratio, (c) spectrum affected by misadjusted water suppression, (d) spectrum acquired without OVS, (e) poorly adjusted B_0 field homogeneity, (f) spectrum affected by uncorrected residual eddy currents

metabolites (in mM concentration range) is extremely difficult in the presence of a large water signal (brain water content ~70–80 %), 4–5 orders of magnitude stronger than the signals of metabolites. The design and properties of pulse sequences most often used for single-voxel ^1H MRS studies of small animals will be discussed in more detail in the following paragraphs. This chapter does not include the methodology of MR spectroscopic imaging (MRSI) [9, 10] due to the lack applications of this technique in animal models of neurodegeneration.

Pulse Sequences for ^1H MRS

The two most frequently used localization pulse sequences for in vivo ^1H MRS are PRESS (point-resolved spectroscopy) [11] and STEAM (stimulated echo acquisition mode) [12]. The PRESS sequence is based on the combination of three orthogonal slice-selection pulses with a double spin echo (90° - 180° - 180°). On the other hand, the STEAM sequence is based on the combination of slice-selective pulses with a stimulated echo (90° - 90° - 90°). Each technique has its pros and cons. The PRESS sequence provides twice as much signal as the STEAM from the same VOI, but the VOI is not as well defined as in STEAM (slice-selection profile of 180° is typically worse than that of 90° pulses). In addition, PRESS is more susceptible for unwanted coherences, and the chemical shift displacement error (see the next paragraph) might be a big problem for high-field ^1H MRS when the peak transmit RF power is limited. The STEAM sequence provides only 50 % of available signal from the VOI (because of the stimulated echo), but the volume selection is superior to PRESS. This sequence is also less susceptible for unwanted coherences that originate from outside of the VOI and enables better water suppression due to an additional water suppression RF pulse during the TM period (between second and third 90° RF pulses). Finally, STEAM allows shorter echo times (TE) than PRESS, which has the advantages of suppressing J -modulation in metabolite spectra with coupled spin systems and detecting signals of metabolites with short T_2 relaxation more reliably. The localization performance of both sequences can be improved by outer volume suppression (OVS) applied prior to the VOI selection (Fig. 2.2a, d), which has been well demonstrated with the ultrashort echo-time STEAM sequence developed for ^1H MRS of rodents at 9.4 T [13].

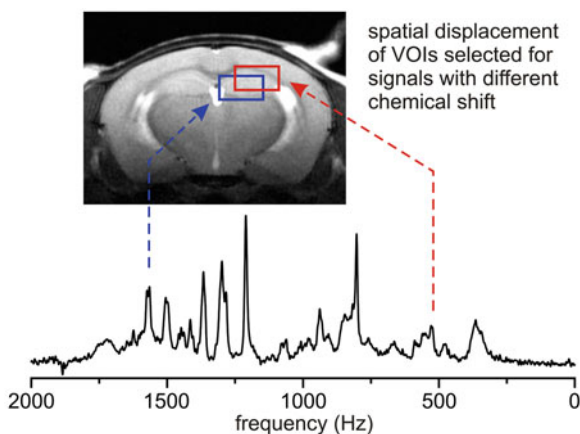
The LASER (localization by adiabatic selective refocusing) pulse sequence [14] provides full intensity from selected VOI similar to the PRESS sequence, but the volume selection, achieved by a pair of adiabatic RF pulses for selection of each slice, is far superior relative to PRESS. In addition, the LASER sequence is fully adiabatic, which means that the VOI is properly selected independent of the applied RF power (above the required adiabaticity threshold). This property of the sequence is extremely useful for MRS studies using surface RF coils with highly heterogeneous transmit B_1 profiles. This advantage in volume selection comes at the cost of increased minimum echo time that might require T_2 correction for absolute metabolite quantification, although TEs as short as 15 ms can be easily achieved with this sequence using standard 9.4 T hardware [7, 15].

More recently, the SPECIAL (spin echo full intensity acquired localization) pulse sequence was introduced [16], which is based on a single spin echo combined with a slice inversion applied on alternative scans (180° - 90° - 180°), similar to 1D ISIS (image-selected in vivo spectroscopy) localization technique [17]. This approach allows acquiring full intensity signal with very short TE. However, these advantages are partially hampered by a potential for spectral contamination resulting from incomplete subtraction of unwanted coherences since SPECIAL is not a single-shot MRS technique.

Chemical Shift Displacement Error

Chemical shift displacement error (CSDE) is a general problem of all single-voxel MRS localization techniques that use slice-selective pulses (simultaneously applied band-selective RF pulse and the field gradient) for VOI selection. CSDE results from a spatial displacement of selected slices for off-resonance signals. It means that metabolite resonances with different chemical shifts originate from volumes that are spatially displaced from the nominal VOI (Fig. 2.3). This spatial displacement is proportional to the ratio of the spectrum frequency range (in Hz) to the RF pulse bandwidth. Most of the signals of interest in ^1H MRS are grouped within a 3 ppm range, but this spectral range corresponds to a frequency range that is linearly proportional to applied magnetic field B_0 . Therefore, CSDE becomes a serious problem at high magnetic fields. For example, the 3 ppm range corresponds to 600 Hz at 4.7 T, but it increases to 1200 Hz at 9.4 T. The commonly used 90° sinc RF pulse of 1 ms has a 3 kHz bandwidth, which results in 20 % VOI displacement for resonances separated by 3 ppm just along one axis at 4.7 T. However, the bandwidth of 180° sinc RF pulse generated by the same peak transmit power is only 2.25 kHz, resulting in 54 % VOI displacement at 9.4 T. This shows the CSDE challenge for the PRESS sequence at high magnetic fields. This drawback in volume selection can be minimized by increasing the bandwidth of RF pulses by

Fig. 2.3 Illustration of the chemical shift displacement error. The volumes from which the metabolite signals originate are displaced from the nominal position of the VOI depending on the frequency offset from the nominal frequency used for the VOI selection



decreasing their duration, as in ultrashort echo-time STEAM [13], or by substituting amplitude-modulated RF pulses by pairs of broadband frequency-modulated adiabatic RF pulses, as it is used in the LASER sequence [14]. These approaches limit the CSDE at 9.4 T to an acceptable 10 % per each slice selection. Although a major CSDE is not recognizable in a spectrum, it may lead to a significant bias and misinterpretation of acquired MRS data.

Water Suppression

Highly effective water suppression is extremely important for reliable quantification of metabolites because the residual water signal, which is rather often out of phase, may cause serious baseline distortions (Fig. 2.2c). Even though the residual water signal can be removed from the spectra using the Hankel singular value decomposition (HSVD) approach [18], this approach cannot eliminate its sidebands (typically caused by gradient coil vibration) overlapping with metabolite resonances. Therefore, in order to minimize unwanted coherences in ^1H MRS data, highly efficient water suppression is always preferred over post-processing water signal removal techniques. The RF pulses applied in the water suppression pulse train have to be frequency selective, suppressing resonances only in a narrow spectral range (± 0.4 ppm) centered on the water resonance at 4.7 ppm. The widely used WET (water suppression enhanced through T_1 effects) technique [19] has been recently more often replaced by the VAPOR (variable pulse power and optimized relaxation delays) water suppression method [13]. This technique, which consists of seven frequency-selective RF pulses, is relatively insensitive to B_1 and T_1 variation and can routinely suppress the residual water signal down to the noise level (Fig. 2.2) [2, 8, 20].

Localization Performance

The localization performance of the sequence can be characterized by two factors: first, how accurately the received FID is localized to the selected VOI and, secondly, to which extent the received FID is contaminated by unwanted coherences from outside of the VOI. The accuracy of localization, outer edges of the VOI, and the CSDE depend on the excitation profiles and bandwidths of the RF pulses utilized in the localization sequence. The small sidelobes on both sides of the central excitation profile can excite spins outside of the VOI. If the sidelobes excite extracerebral subcutaneous tissue, unwanted signal from lipids (around 1.5 ppm and typically out of phase) will contaminate the ^1H MRS spectrum of the brain (Fig. 2.2d). These imperfections in the localization performance can be minimized by appropriate OVS (Fig. 2.2a, d). The OVS scheme must utilize broadband RF pulses (the frequency-modulated hyperbolic secant pulses are the best choice) to keep the CSDE of OVS in a similar range as the CSDE of the VOI.

The second challenge that accompanies the volume selection originates from the fact that the series of RF pulses also creates other types of coherences (FIDs, spin

and stimulated echoes) that have to be eliminated by crusher gradients (gradient dephasing) and phase cycling of RF pulses and receiver. Unwanted coherence suppression by gradient dephasing is preferable because the elimination of strong unwanted coherences by subtraction does not work reliably under in vivo conditions. The dephased magnetization is apparently lost. However, this apparently lost magnetization can be rephased back during signal detection by inhomogeneous B_0 fields outside of the VOI and can generate unwanted echoes.

Because of an inherently low detection sensitivity of MRS and strong overlap of metabolite resonances in an overcrowded spectral range (1–4 ppm), the spectral quality is critical for reliable metabolite quantification. Unwanted signals, deteriorating the spectral quality, always originate from outside of the VOI. A sufficient SNR (Fig. 2.2a, b) is very important for accurate and precise metabolite quantification. But the quality of the spectra, which depends on B_0 shimming, the efficiency of water suppression, and the elimination of unwanted coherences, is even more important than the SNR (Fig. 2.2). Only well-designed and optimized pulse sequences can provide high-quality spectra, which are critical for meaningful analysis and neurochemical profiling. There are many examples in literature for high-quality ^1H MR spectra measured by STEAM [2, 8, 20–22], LASER [6, 7, 15, 23], and SPECIAL [24–26] pulse sequences.

Eddy Currents

Eddy currents are induced in the conducting structures of the magnet by fast magnetic field gradient switching and result in undesirable time-varying magnetic field gradients and magnetic field shifts. These gradients and field shifts cause line broadening and distortions of lineshape. Despite compensation for eddy current effects by hardware, some residual eddy currents always persist and affect spectral linewidth and lineshape (Fig. 2.2f) and thereby may cause major problems in accuracy of metabolite quantification.

Animal Handling and Data Acquisition Strategies

Successful in vivo ^1H MRS requires keeping optimal and stable physiological conditions during the entire experiment. To achieve that, a well-designed animal holder, which firmly holds the rat or mouse head in stable position and at the same time allows smooth respiration, is required. Continuous monitoring of respiration and body temperature is essential. The respiration rate for spontaneously breathing mice should be kept in a range of 80–100 per minute by adjusting the isoflurane level in anesthetic gas mixture ($\text{N}_2\text{O}/\text{O}_2 \sim 1:1$). The deoxyhemoglobin is a paramagnetic molecule and is a major source of microscopic B_0 heterogeneity of the tissue, which affects the signal linewidth. Without optimal blood oxygen saturation, high spectral resolution is not possible despite perfect B_0 shimming.

Animal respiration and small body motions (not the head) result in frequency fluctuations, which cause line broadening if these fluctuations are not corrected. Even very small head movements, caused, e.g., by gasping, result in signal phase fluctuations, which decrease the signal intensity of the averaged spectrum. Therefore, a single-scan averaging mode (FID of the each scan is saved separately in memory) is preferred in order to allow post-processing phase and frequency corrections before data summation. If the SNR of a single-scan spectrum is too low (VOI is too small) for performing any correction, then data acquisition in small blocks is recommended. For example, instead of acquiring 160×1 scan, 20×8 scans can be acquired. This approach enables frequency correction at least between these 20 FIDs (spectra) before summation. In addition, this MRS data collection approach allows eliminating corrupted blocks of data from summation to avoid deterioration of the final spectral quality due to intermittent instability.

MRS Data Processing and Quantification

MRS Data Pre-processing

Frequency and Phase Correction

As it was mentioned before, respiration and small body movements can cause frequency and phase fluctuations in the acquired data. In addition, maintaining a long-term stability of the B_0 field within ± 1 Hz is difficult. Therefore, a single-scan averaging approach is highly recommended because it allows correction of any frequency or phase change on each single scan (FID). Preferentially, these corrections have to be applied on time-domain data (FIDs). If the SNR of single-scan data is not sufficient, then the single-scan data should be first summed in small blocks (e.g., eight scans per block) and then the frequency correction can be applied on these summed data. This approach cannot correct any phase or frequency instability that occurs within these short time periods, but can efficiently remove a long-term frequency drift. Uncompensated small frequency fluctuations result in broader lines, but signal integrals are preserved. However, uncompensated phase fluctuations reduce the signal integrals and may lead to an underestimation of metabolite concentrations. Therefore, blocks with obviously decreased intensity must be eliminated from the final summation. If we already know that the SNR of single-scan data will not be sufficient to perform these corrections, saving data acquired in relatively short time periods (e.g., eight scans) is a reasonable approach.

Correction for Residual Eddy Currents

Lineshape distortions caused by residual eddy currents can be easily removed from metabolite spectra using characteristics of the lineshape distortion taken from the

unsuppressed water signal [27]. This unsuppressed water signal must be acquired exactly with the same set of parameters, including all gradients, as those used for metabolite spectra acquisition, except for RF pulses used for water suppression. This eddy current correction can be applied on individual scans or simply on the final summed FID.

Metabolite Quantification

Analysis of short echo-time in vivo ^1H MR spectra of the brain is rather complex and requires sophisticated fitting methods. Despite increased chemical shift dispersion at ultrahigh magnetic fields and optimal B_0 shimming, spectra of individual metabolites are highly overlapped. Therefore, a simple integration method that has been routinely used for long TE spectra with three or four peaks is not applicable for short TE spectra. A big advantage for meaningful quantification of ^1H MRS spectra is the knowledge about metabolites that make a dominant contribution to in vivo spectrum of the brain. The homeostasis of the brain is extremely well maintained, therefore except in cases of major metabolic defects, the set of metabolites contributing to brain spectra is well defined. In addition, the pH and temperature of the brain are typically maintained at a very narrow range, which means that ^1H MR spectra of metabolites can be easily measured under identical conditions. The database of these metabolite spectra forms the prior knowledge for all spectral quantification techniques. In order to get meaningful neurochemical information, fitting algorithms decompose the in vivo spectrum into appropriately broadened metabolite spectra from the database. It is a rather simplified model, but it works reasonably well.

Quantification Methods

The two most commonly used spectral fitting software packages are jMRUI [28, 29], working in the time domain, and LCModel [30], working in the frequency domain. If the same prior knowledge is used, both methods should provide very similar quantification results [31]. Quantification errors are estimated by Cramér-Rao lower bounds (CRLB). It should be emphasized that CRLB are estimated on the basis of the assumption that the model (spectral basis set) is correct and complete. Obviously, this is not possible and reasonable simplifying assumptions have to be made. The metabolite spectra included in the database can be experimentally measured or simulated based on published information about metabolite chemical shifts and J -couplings [32]. The basic principle of LCModel analysis is demonstrated in Fig. 2.4. The in vivo ^1H MR spectrum (also shown in Fig. 2.1a) is decomposed into a linear combination of metabolite spectra from the prior knowledge database (basis set). The fitting routine is scaled in such a way that the coefficients of this linear combination are the estimated metabolite concentrations.

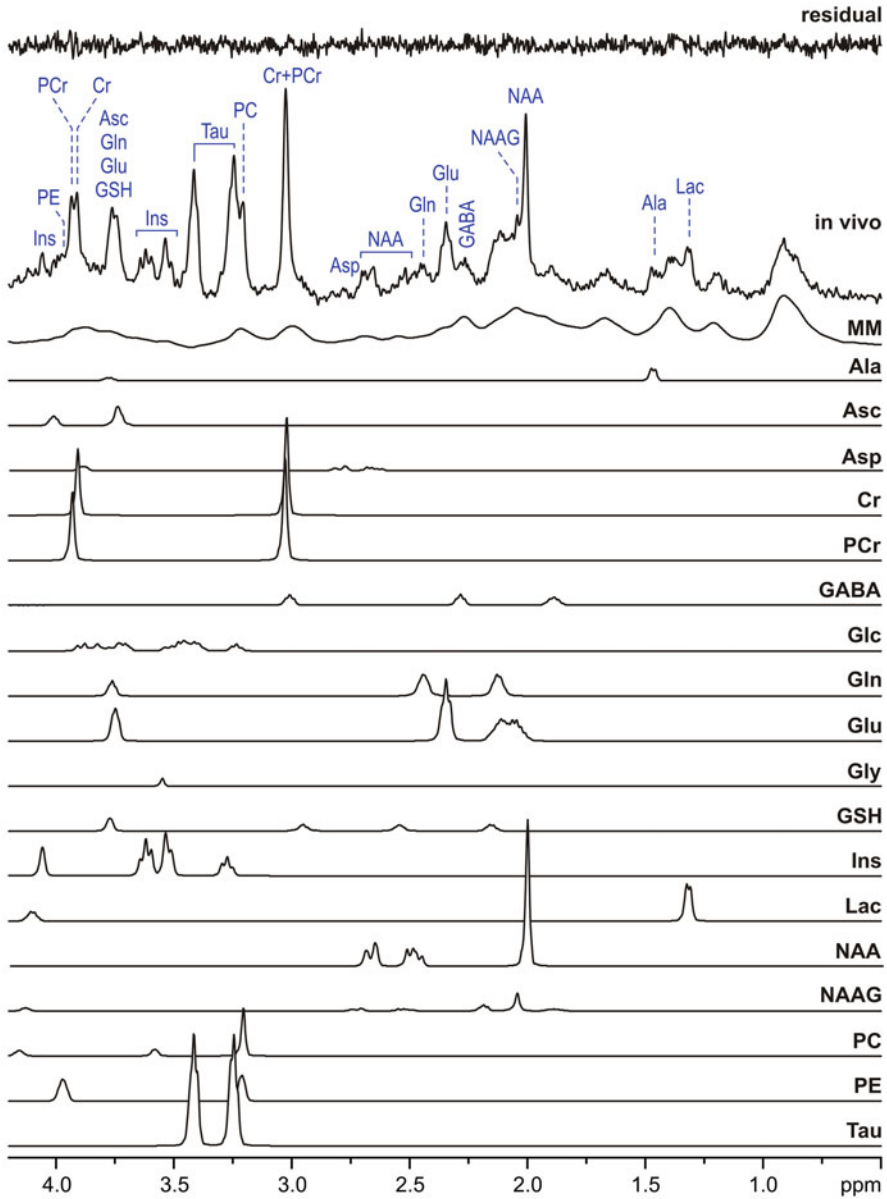


Fig. 2.4 LCModel analysis of the spectrum acquired from the mouse hippocampus at 9.4 T (shown on Fig. 2.1). LCModel analysis was performed using a simulated basis set that includes experimentally measured spectrum of fast-relaxing macromolecules

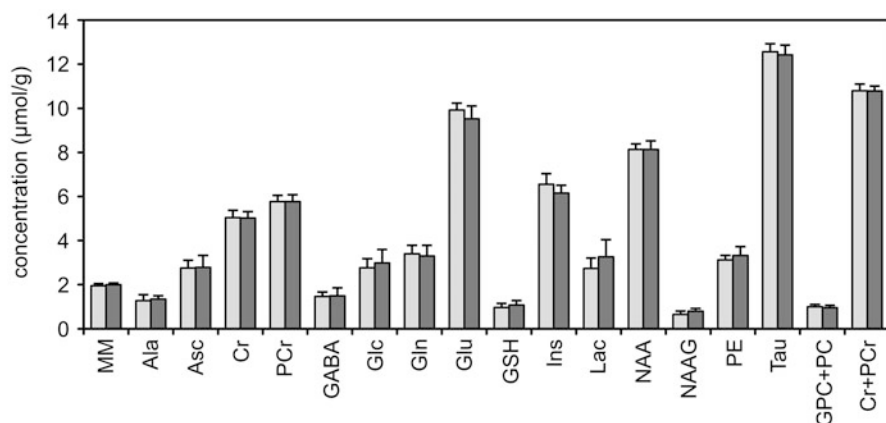


Fig. 2.5 Reproducibility of neurochemical profiling in the mouse brain at 9.4 T. The neurochemical profiles of the hippocampus measured from two different groups of male C57BL/6 mice ($N = 10$ in each group) are shown in *light* and *dark gray*. Error bars indicate the SD

The result of the LCModel analysis is shown as a bar diagram in Fig. 2.1. The reproducibility of the neurochemical profiling in mice is demonstrated in Fig. 2.5, where the metabolite concentrations quantified from the hippocampus of two different groups of C57BL/6 mice are compared.

Macromolecule Background

Short TE ^1H MR spectra have significant signal contribution from fast-relaxing macromolecules that are easily recognizable between 0.5 and 1.8 ppm (Figs. 2.1 and 2.4). A dominant contribution to these macromolecule signals in the healthy brain comes from mobile proteins in cytosol. These broad signals must be taken into account in metabolite quantification and should not be fitted as a baseline. Including a measured macromolecule spectrum in the basis set is a very robust approach, which improves the quantification of weakly represented metabolites, such as GABA [33, 34]. The macromolecule spectrum can be experimentally measured using an inversion-recovery experiment [34]. The small residual metabolite signals can be suppressed using diffusion weighting [35], or they can be removed from the spectra in post-processing [36].

Referencing

Metabolite quantification requires appropriate referencing to obtain values in concentration units. The signal of total creatine (Cr + PCr) has been widely used as an internal reference, assuming a concentration of 8 $\mu\text{mol/g}$. However, using total creatine as an internal reference is far from being optimal because its content varies

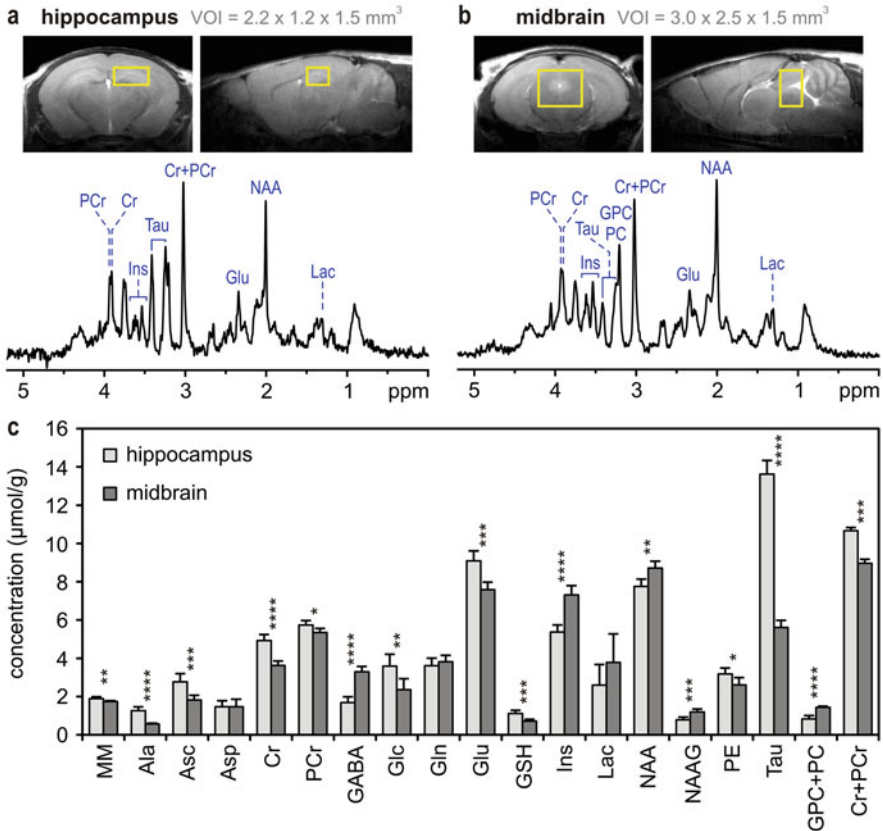


Fig. 2.6 Comparison of the neurochemical profiles acquired from the hippocampus and midbrain of C57BL/6 mice ($N = 6$) at 9.4 T. Error bars indicate the SD, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$

between brain regions (Fig. 2.6) and changes significantly during brain development [24, 25, 37] and as a consequence of neurodegenerative processes [20, 21]. Using the unsuppressed water signal as a reference is a very useful approximation and has worked extremely well in many in vivo ¹H MRS applications [1, 6–8, 15, 20, 21, 23, 26]. An alternative referencing method called ERETIC (Electric Reference To access In vivo Concentrations) has been described [38].

Relaxation

It is extremely difficult to precisely measure T_1 and T_2 relaxation times of all brain metabolites in vivo. In addition, relaxation times are not properties of molecules, but each proton in a molecule has its own T_1 and T_2 values. The relaxation parameters of metabolites have been recently assessed based on the simplified

assumption that all CH₂ and CH protons within the same molecule have approximately the same relaxation properties [39–41]. When MRS data are acquired using shorter repetition time (TR) or longer TE, then corrections for relaxation effects must be applied for “absolute” metabolite quantification. But these corrections are unnecessary when an ultrashort TE localization sequence and a long TR are used [8, 20–22], making this a robust approach for reliable neurochemical profiling.

Neurochemical Profiling

The primary goal of neurochemical profiling is to extend the range of quantifiable metabolites and to improve the precision and the accuracy of their quantification. Reliable quantification of more than 15 brain metabolites is feasible from the rodent brain when advanced high-field MR instrumentation, carefully optimized pulse sequences and acquisition strategies, and sophisticated data processing methods are used [1, 6–8, 15, 20–22, 26, 33]. The metabolite distribution in the rat or mouse brain is rather heterogeneous, which is illustrated in Fig. 2.6 where the neurochemical profiles of the hippocampus and the midbrain are compared. This figure also demonstrates the power of ¹H MRS to detect differences in tissue composition.

In conclusion, reliable, noninvasive quantification of an extended range of brain metabolites in rodents is feasible. To achieve this goal, high-quality *in vivo* ¹H MR spectra and sophisticated processing tools with well-optimized prior knowledge are needed and are now being used successfully by an increasing number of laboratories worldwide.

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