Ultra-high field MRS of rodents

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Rats and mice may serve as subjects for modeling various pathological conditions, which can be studied by localized MR spectroscopy. In contrast to MR imaging, which relies on properties of water related to its interaction with biomacromolecules, spectroscopy is capable of providing information on concentrations of chemical entities present in a living tissue.

Measurements at high magnetic fields benefit from the higher signal-to-noise ratio (SNR) and increased spectral dispersion, which improves quantification accuracy and precision. The benefits are expected to be important especially for metabolites having low concentration or overlapping spectral lines and for compounds giving complex J-coupled spectral patterns. These advantages, however, are partially offset by a decrease of T_2* with increasing static magnetic field [1-3].

The increase of spectral dispersion at ultra-high magnetic fields is particularly useful for proton (^{1}H) MRS of rodent brain, which is complicated by overlapping peaks of a number of metabolites [4]. Fig. 1 compares proton MR spectra of rat brain measured at 9.4 Tesla and at 14.1 Tesla [5]. Two novel features can be seen at 14.1 T, in particular, a group of spectral lines resonating between 4.2 and 4.4 ppm, and the narrowing (in ppm) of the spectral lines of GABA, glutamate and N-acetylaspartate in the spectral range from 1.8 ppm to 2.6 ppm. The improved spectral dispersion enabled to discern several low-intensity peaks in the spectral region from 3.5 ppm to 4.2 ppm, which were assigned to lactate (4.11 ppm), glucose (3.85 ppm) and glycerophosphocholine (3.67 and 3.87 ppm). The resolved resonances at 4.31 and 4.27 ppm, which typically are saturated at lower fields by water suppression pulses, and additional peaks/peak shoulders of GPC resolved between 3.6 ppm and 3.9 ppm may substantially improve the accuracy of the GPC and phosphocholine

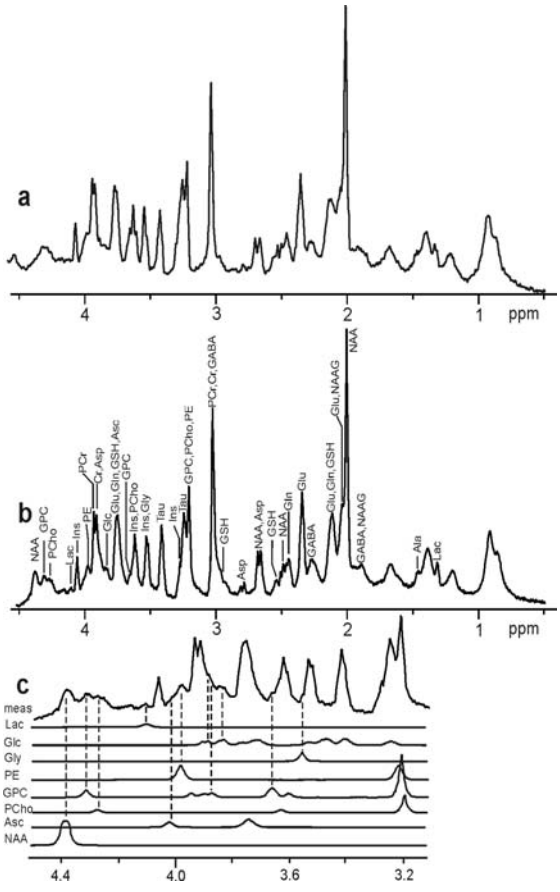

Fig.1. 9.4 T (**a**) and 14.1 T (**b**) proton MR spectrum of a rat brain. The expanded region (**c**) shows assignment of small peaks in the 3.2 – 4.4 ppm region.

Table 1
Mean metabolite concentrations (±standard deviations) and mean CRLB calculated from five pairs of spectra measured at 9.4 T and 14.1 T, respectively

Metabolite	9.4 T		14.1 T	
	Concentration (mmol/kg) ± SD	CRLB (%)	Concentration (mmol/kg) ± SD	CRLB (%)
Alanine (Ala)	0.37 ± 40%	23	0.63 ± 19%[a]	11
Aspartate (Asp)	1.7 ± 31%	14	1.9 ± 10%	9
Phosphocholine (PCho)	0.47 ± 33%	16	0.32 ± 16%[a]	16
			0.49 ± 26%[b]	17
Glycerophosphocholine (GPC)	0.33 ± 30%	18	0.87 ± 5%[a]	6
			0.66 ± 32%[ab]	12
Creatine (Cr)	3.9 ± 12%	4	4.0 ± 11%	3
Phosphocreatine (PCr)	4.5 ± 9%	3	4.3 ± 10%	3
γ-Aminobutyrate (GABA)	1.1 ± 22%	9	1.5 ± 13%[a]	6
Glutamine (Gln)	3.0 ± 19%	3	2.8 ± 19%	3
Glutamate (Glu)	9.8 ± 6%	1.5	10.3 ± 8%	1.2
Glutathione (GSH)	1.0 ± 12%	7	1.3 ± 14%[a]	5
Glycine (Gly)	0.50 ± 32%	22	0.81 ± 17%	13
Glucose (Glc)	1.2 ± 66%	30	2.3 ± 10%[a]	9
myo-Inositol (Ins)	5.9 ± 6%	2	6.2 ± 5%	2
N-Acetylaspartate (NAA)	9.2 ± 10%	1.0	9.3 ± 12%	1.0
Taurine (Tau)	6.1 ± 12%	2	6.0 ± 9%	2
Ascorbate (Asc)	1.2 ± 40%	14	1.4 ± 33%	11
N-Acetylaspartylglutamate (NAAG)	0.40 ± 51%	6	0.96 ± 16%[a]	6
Phosphoethanolamine (PE)	2.1 ± 9%	6	2.2 ± 12%	9
Lactate (Lac)	1.4 ± 33%	7	0.73 ± 19%[a]	12

The spectra in each pair had the same SNR (within ±3%), the overall range of SNR was 27–40.

[a] Metabolite concentrations, which were found significantly different at 14.1 T compared to 9.4 T ($t < 0.1$).

[b] Only the spectral range from 0 to 4.1 ppm was used in the LCModel fit.

quantification. Table 1 summarizes 19 metabolites, which were quantified at ultra-high fields. Relative standard deviations and Cramer-Rao lower bounds of metabolite concentrations are nearly the same or lower at 14.1 T than at 9.4 T.

The detection of ^{13}C label incorporation in conjunction with ^{13}C-labeled substrate administration is frequently done by combining three-dimensional localization with ^{1}H-observed-^{13}C-edited spectroscopy, which is based on inverting the magnetization of protons bound to ^{13}C on alternate spin-echo scans [6,7]. The ultra-high field is also useful for these experiments, in particular for separate quantification of ^{13}C-labeled Glu and Gln (Fig. 2).

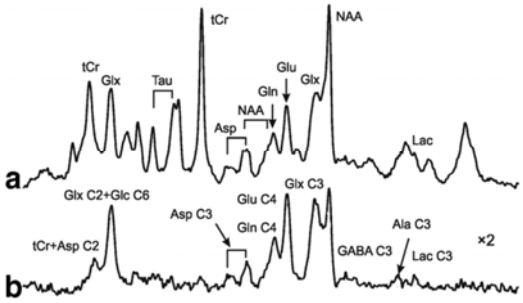

Fig. 2. (**a**) ^{1}H spectrum of the rat brain in at 9.4 T. (**b**) ^{1}H-[^{13}C] spectrum using ACED-STEAM [8]. *Courtesy of L. Xin.*

The increased sensitivity at a high magnetic field is also beneficial for proton spectroscopic imaging (SI) of rodent brain. Since the brain of rodents is relatively small, measuring spectra from small brain structures or lesions by high-resolution SI is a feasible alternative to localized single voxel spectroscopy. Fig. 3 shows spectra of "pure" hippocampus and corpus callosum obtained by summing spectra from 6 voxels (a nominal voxel size of 1.1 uL) of the short-echo-time SI scan of rat brain [9]. This technique reduces chemical shift artefacts and can shorten the total measurement time, when spectra from different brain regions are to be measured. The quality of spectral information can be affected by the B_0 field homogeneity, which may not be optimal in all voxels of interest, and by the chemical shift displacement error near edges of an excited region.

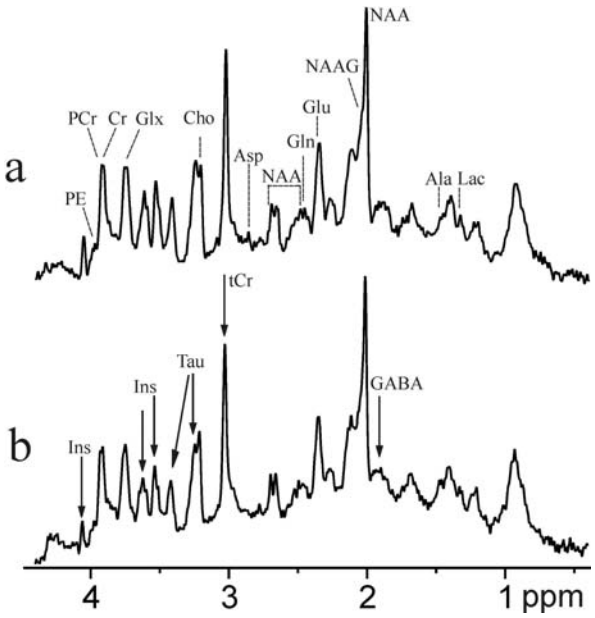

Fig. 3. Spectra of "pure" hippocampus (**a**) and corpus callosum (**b**) obtained by summing six voxels in each structure. Note the decreased concentrations of GABA, total creatine, taurine and myo-inositol in (**b**).

Nuclei other than hydrogen usually provide spectra with a higher chemical shift dispersion. On the other hand, they are less sensitive and usually less abundant than protons so that an increase of sensitivity at ultra-high magnetic field is highly desirable. In vivo ^{13}C magnetic resonance spectroscopy is usually performed in conjunction with administrating ^{13}C substrates. ^{13}C NMR studies were used for measuring label incorporation into various metabolites [10,11] and they showed improved resolution at ultra-high magnetic fields. The ^{13}C spectroscopy can be used for the detection of not only low-molecular-weight metabolites but also for biomacromolecules such as glycogen. Fig. 4 shows a localized ^{13}C spectrum of C_1 of glucose and glycogen in rat brain using a SIRENE pulse sequence (3D outer volume saturation, 1D ISIS and 1D inversion nulling [12]). The nearly constant linewidths (in Hz) at 9.4 T and 14.1 T together with the increased spectral dispersion resulted in a more defined baseline at 14.1 T, which allowed better spectral peak fitting and quantification.

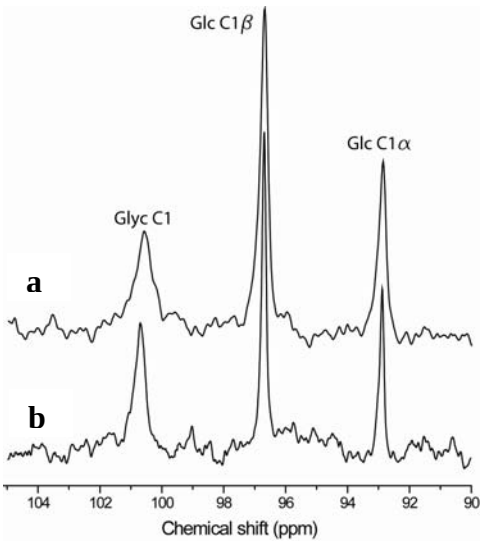

Fig. 4. A comparison of ^{13}C MR signals of glucose and glycogen generated at 9.4 T(**a**) and 14.1 T(**b**). *Courtesy of RB. van Heeswijk.*

The high static magnetic field and the corresponding increase in sensitivity is also useful for ^{15}N spectroscopy experiments monitoring ^{15}N

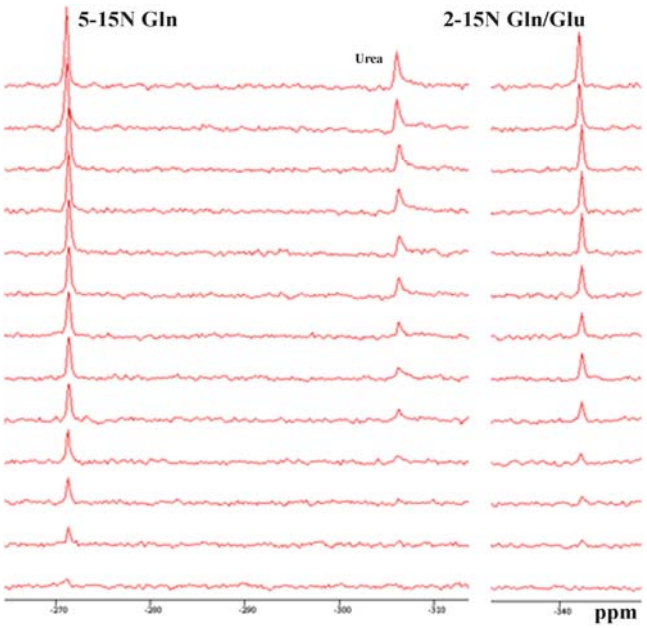

Fig. 5. A series of ^{15}N spectra of rat brain acquired at different time points for up to 9h of $^{15}NH_4Cl$ infusion. *Courtesy of C. Cudalbu.*

label incorporation. Fig. 5 shows ^{15}N spectra of rat brain at 9.4 T during ^{15}N-labeled ammonia infusion. The first ^{15}N peaks were detected as early as 25 and 50 minutes, respectively, after starting infusion.

In summary, the ultra-high magnetic field is advantageous for in vivo proton or heteronuclear MR spectroscopy since it provides higher SNR, improves spectral resolution and reduces effects of spin-spin coupling. These features enable to monitor nearly 20 metabolites in proton spectra of rodent brain and to improve precision and reliability of metabolite quantification in a great variety of biological experiments.

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