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SCIENCE DIRECT
Magnetic Resonance Imaging 24 (2006) 343–348
MAGNETIC RESONANCE IMAGING

Sensitivity of single-voxel ^{1}H-MRS in investigating the metabolism of the activated human visual cortex at 7 T

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Received 2 December 2005; accepted 2 December 2005

Abstract

Proton magnetic resonance spectroscopy (^{1}H-MRS) has been used in a number of studies to noninvasively assess the temporal changes of lactate in the activated human brain. However, the results have not been consistent. The aim of the present study was to test the sensitivity of ^{1}H-MRS during functional experiments at the highest magnetic field currently available for human studies (7 T). Stability and reproducibility of the measurements were evaluated from LCModel analysis of time series of spectra measured during a visual stimulation paradigm and by examination of the difference between spectra obtained at rest and during activation. The sensitivity threshold to detect concentration changes was 0.2 μmol/g for most of the quantified metabolites. The possible variations of metabolite concentrations during visual stimulation were within the same range (±0.2 μmol/g). In addition, the influence of a small line-narrowing effect due to the blood oxygenation level-dependent (BOLD) T2* changes on the estimated concentrations was simulated. Quantification of metabolites was, in general, not affected beyond 1% by line-width changes within 0.5 Hz.

Keywords: Single-voxel proton spectroscopy; Ultrahigh magnetic field; BOLD; Lactate

1. Introduction

Proton magnetic resonance spectroscopy (^{1}H-MRS) has emerged as a powerful tool to investigate the metabolism of the human brain during activation. The detection of functional changes of metabolite concentrations may help to understand the metabolism that sustains the brain at work. So far, single-voxel ^{1}H-MRS as well as spectroscopic imaging, generally performed at magnetic fields lower than 3 T, have been used with the goal of identifying variations of metabolites during several kinds of brain activation [1–11]. In particular, lactate (Lac) has been receiving special attention, since the functional involvement of this metabolite was firstly hypothesized after the study conducted in 1988 by Fox et al. [12], who found an uncoupling between oxygen and glucose consumption during long stimuli. Lactate, and not glucose, was later proposed by Pellerin and Magistretti [13] as the main metabolic substrate for *activated* neurons, in the framework of the so-called astrocyte–neuron Lac shuttle. Lactate was reported by some authors to increase during prolonged visual stimulations [1,2,5], during a motor task [4] and during silent word generation [7]; a decrease was also observed few seconds after the presentation of an impulsive visual stimulation [6]. The results of these studies have been inconsistent thus far; for example, long photic stimuli were reported to cause either 50–60% [1,5] or 250% [2] increases in Lac concentration. These discrepancies were probably due to different experimental parameters adopted in the various laboratories and to the fact that Lac is a low-concentration metabolite (about 1 μmol/g) and therefore its reliable detection in basal condition was not always possible. In fact, the feasibility itself of detecting Lac changes has been challenged by some authors: a high variability of the basal

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doi:10.1016/j.mri.2005.12.023

level of Lac was underlined by Merboldt et al. [3] who did not detect any reproducible time course between subjects. More recently, Boucard et al. [8] did not observe any significant alteration of the spectra during prolonged stimulation; in their setup, the region of the spectrum around 1.33 ppm was indeed affected by unstable signals presumably coming from lipids of the scalp. The authors concluded that previous claims about Lac changes might have an artefactual origin.

Optimizing the sensitivity and accuracy of the methodology for functional MRS studies is important not only because metabolites are present in low concentration, but also because concentration changes are expected to be relatively small, since the brain metabolite concentrations are likely to be homeostatically controlled in physiological conditions. In this context, ultrahigh magnetic field systems can help in obtaining robust and accurate time courses of metabolites, due to the increased spectral dispersion and signal-to-noise ratio (SNR) compared to lower fields [14].

In the present study we investigated the sensitivity of single-voxel ^{1}H-MRS at 7 T for functional applications, with the purpose of establishing a threshold limit of concentration changes that can be detected with statistical certainty. A high number of metabolite concentrations was quantified during a visual stimulation paradigm, similar to the one previously used by Frahm et al. [5].

It has been previously reported that the blood oxygenation level-dependent (BOLD) effect produces a small line-narrowing (around 0.2–0.3 Hz) on the spectra at 4 T [15]. A secondary aim of the study was to determine the influence of the BOLD effect on the quantification of metabolites obtained by LCModel [16] at 7 T.

2. Methods

The measurements described herein were performed on a 7 T/90 cm magnet (Magnex Scientific, UK), interfaced to Varian INOVA console. The system was equipped with a head gradient coil (40 mT/m, 500 μs rise time) and strong custom-designed second-order shim coils (Magnex Scientific) with the maximum strengths of $XZ=5.8\times10^{-4}$ mT/cm^2, $YZ=5.6\times10^{-4}$ mT/cm^2, $Z^2=9.0\times10^{-4}$ mT/cm^2, $2XY=2.8\times10^{-4}$ mT/cm^2 and $X^2-Y^2=2.9\times10^{-4}$ mT/cm^2 at a current of 4 A. All first- and second-order shim terms were automatically adjusted using FASTMAP with EPI readout [17,18]. In vivo ^{1}H-NMR spectra were acquired using ultrashort echo-time STEAM (TE=6 ms, TM=32 ms, TR=5 s) optimized for applications in humans at ultrahigh magnetic field [19]. A "double localization" was performed with STEAM and four modules of outer volume saturation; water signal was suppressed by VAPOR [20,21].

Two healthy volunteers gave informed consent according to procedures approved by the institutional review board and the FDA. Each subject was investigated twice during a paradigm of visual activation, with the voxel of interest (VOI=20×20×20 mm^3) positioned inside the visual cortex in one case and outside in the other (control conditions). The stimulus, which was projected to a mirror fixed on the head coil, consisted of a radial red/black checkerboard covering the entire visual field and flickering at a frequency of 8 Hz. A red cross in the middle of the image was used as fixation point; in order to check their attentional status, the volunteers were asked to press a button whenever the cross in the fixation point changed orientation.

Initial fMRI sessions based on BOLD contrast were performed before the spectroscopy studies in order to identify the activated visual area. The parameters used were single-shot gradient-echo echo planar imaging (GE-EPI), 16 sagittal slices, TE=22 ms, spatial resolution=2.5×2.5×2.5 mm^3, TR=2.5 s; functional paradigm: eight trials of 10 s ON+22.5 s OFF. Cross-correlation (cc) coefficients were calculated pixelwise between a hemodynamic reference waveform and the fMRI time series. Only pixels with cc≥0.3 were considered activated, and a cluster filter (cluster size ≥6 contiguous pixels) was applied to produce final activation maps.

The protocol of functional spectroscopy involved initial 32 scans at rest (black image) and four periods (64 scans each, 5.3 min long) acquired in an interleaved manner during conditions of visual stimulation ON and OFF, for a total duration of about half an hour. This was a study duration that ensured reasonable stability, attention and comfort of the volunteer.

After having applied frequency and phase corrections on single scans, nine spectra (32 scans each) were obtained. These spectra were corrected for residual eddy currents by using internal water reference and finally they analyzed by LCModel [16]. The unsuppressed water signal measured from the same VOI was used as an internal reference for quantification assuming brain water content of 80%. The LCModel basis set included the simulated spectra of 21 metabolites and the spectrum of fast relaxing macromolecules experimentally measured from the human brain using an inversion-recovery experiment (TR=2 s, IR=0.675 s) [21]. Those metabolites that were quantified with Cramer–Rao lower bounds (CRLBs) >30% were discarded for further analysis.

In order to test the influence of line-width changes (resulting from the BOLD effect) on metabolite quantification, FIDs were multiplied by exponential functions corresponding to 0.1–0.5-Hz line broadening and then fitted with LCModel. Appropriate white noise was added back to FIDs to keep the noise level constant.

3. Results

Spectra obtained during rest and stimulation conditions from the same subject, with the voxel localized inside and outside the visual cortex, are shown in Fig. 1. Shimming resulted in water line widths around 13–14 Hz, with concomitant creatine (Cr) line widths of 11–12 Hz. Spectra were highly reproducible between different sessions and

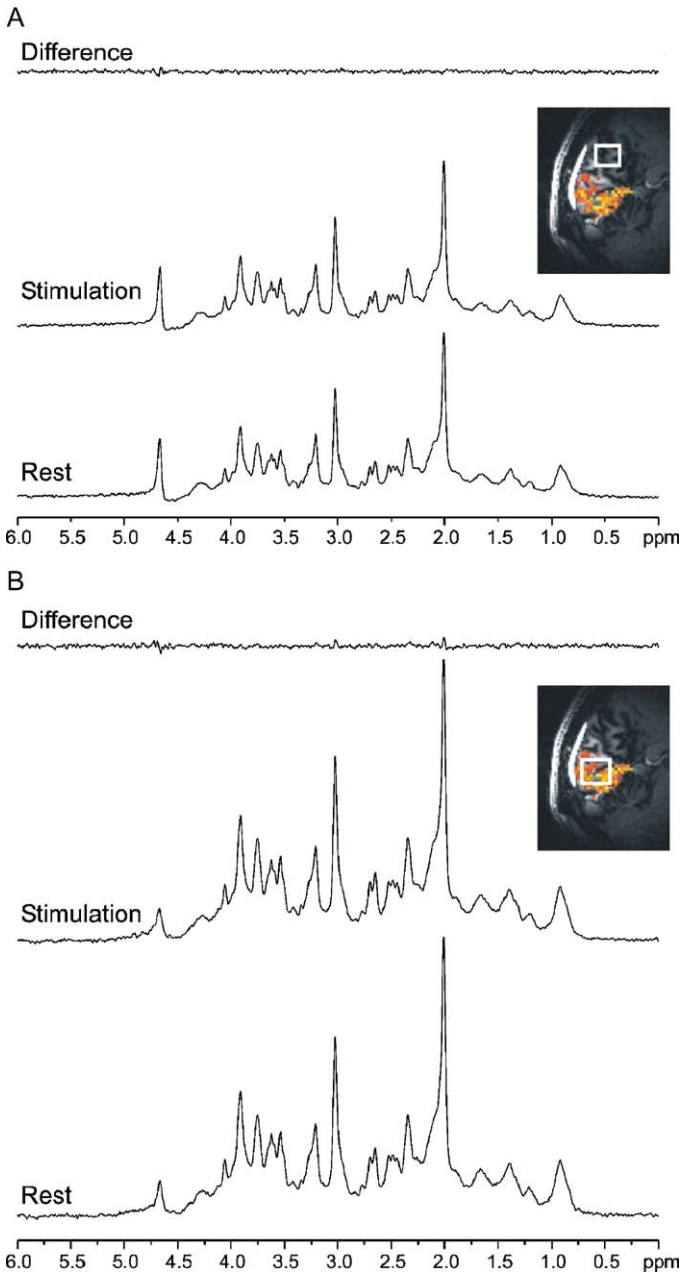

Fig. 1. Spectra obtained at rest and during stimulation in a single subject, when the VOI ($20\times20\times20$ mm^3) was located outside (A) and inside (B) the visual cortex. The insets depict functional maps with the localization of the VOIs (GE-EPI, TE = 22 ms, TR = 2.4 s, spatial resolution $2.5\times2.5\times2.5$ mm^3; activated pixels correspond to cc ≥ 0.3, cluster size ≥ 6 contiguous pixels). Differences between spectra obtained in the two conditions of rest and stimulation are also shown. Spectroscopic parameters: STEAM, TE = 6 ms, TM = 32 ms, TR = 5 s. Processing: frequency and phase corrections of individual scans, summation of 32 scans, residual eddy currents correction, gaussian multiplication ($\sigma = 0.0865$ s), FFT and zero-order phase correction. No further postprocessing, such as water signal removal and baseline correction, was performed. Spectra in A and B have the same vertical scale. The small narrow peaks at 2.0 and 3.0 ppm, visible in the difference spectrum when the VOI was located inside the visual cortex, were ascribed to the BOLD effect.

different subjects. Localization performance of the sequence and efficient VAPOR water suppression resulted in spectra with minimal distortions and a flat baseline in the entire chemical shift range. Contamination by signals from extracerebral lipids was not observed in spite of the ultrashort TE of 6 ms.

The difference between spectra obtained at rest and during stimulation (Fig. 1) revealed minimal residuals above the noise level; when the voxel was located inside the visual cortex (Fig. 1B), narrow small peaks (line width around 6 Hz) were visible in the difference spectra at the positions of the singlets of *N*-acetylaspartate (NAA) (2.0 ppm) and Cr (3.0 ppm). This effect was not present when the voxel was located outside the visual cortex (Fig. 1A). With the achieved SNR, no other peaks were evident in the difference spectra.

Table 1
Quantification of metabolite concentrations by LCModel

	Concentration (μmol/g)	CRLB (μmol/g)	CRLB (%)	S.D., voxel OUT (%)	S.D., voxel IN (%)	Simulated BOLD effect (%)
Asc	1.2	0.21	18	11	6	3.1
Asp	1.0	0.26	28	15	16	3.8
Cr	5.0	0.26	5	3	2	0.8
PCr	3.4	0.24	7	4	3	1.4
GABA	1.0	0.13	14	4	9	1.7
Glc	1.1	0.28	25	13	17	0.3
Gln	2.9	0.16	6	4	3	0.1
Glu	11.0	0.22	2	1	1	1.1
GSH	1.0	0.10	10	8	7	0.9
Ins	6.7	0.20	3	1	2	1.0
Lac	0.8	0.10	13	8	19	0.1
NAA	10.8	0.14	1	1	1	1.2
NAAG	1.4	0.11	8	3	5	−0.4
PE	1.2	0.19	16	12	7	−1.4
Scyllo	0.4	0.05	12	9	4	2.4
Tau	1.9	0.18	10	4	4	3.4
GPC+PCho	1.3	0.05	4	2	1	1.4

Values were calculated from all nine spectra obtained during each functional study and by averaging the data from the two subjects. Voxel OUT or IN means outside (control conditions) or inside the visual cortex. The column relative to "Simulated BOLD effect" indicates the estimated artefactual concentration changes derived by LCModel at 0.4 Hz line narrowing.

Concentrations of ascorbate (Asc), aspartate (Asp), Cr, phosphocreatine (PCr), glucose (Glc), glutamate (Glu), glutamine (Gln), glutathione (GSH), inositol (Ins), Lac, NAA, *N*-acetylaspartylglutamate (NAAG), γ-aminobutyric acid (GABA), scyllo-inositol (Scyllo), taurine (Tau), choline, and phosphorylethanolamine (PE) were quantified by LCModel with CRLB <30% (Table 1), which corresponded to CRLB below 0.2 μmol/g for most of the quantified metabolites in each individual study. In particular, the average CRLB of lactate was ~0.1 μmol/g, amounting to 10% of uncertainty. In order to assess the reproducibility of the measurements, intrasubject variations (expressed as S.D.) were calculated in control conditions, that is when the voxel was located outside the visual cortex. Concentration variations were always slightly lower than the average CRLB for all metabolites (Table 1). No discernible trend with time and stimulus onset was observed (Fig. 2A). When the voxel was located inside the visual cortex (Fig. 2B), the time courses obtained with LCModel revealed that concentration changes of all quantified metabolites were within ±0.2 mol/g. Also in this case, S.D.'s were lower than CRLB, except for lactate whose S.D. was more than twofold higher than the S.D. in control conditions (Table 1).

Simulations demonstrated that when applying line broadening up to 0.5 Hz, estimates of all metabolite concentrations were reproducible within the limit imposed by the CRLB of the fit (Fig. 3). For most of the quantified metabolites the estimated concentrations systematically decreased by almost 1% when increasing the line width by 0.3–0.4 Hz, that is on the same order of the expected line narrowing due to the BOLD effect at 7 T (Table 1). Asc, Asp, Scyllo and Tau were slightly more influenced by the introduced line narrowing (2–4%). In contrast, quantifications of Glc (0.3%), Gln (0.1%) and

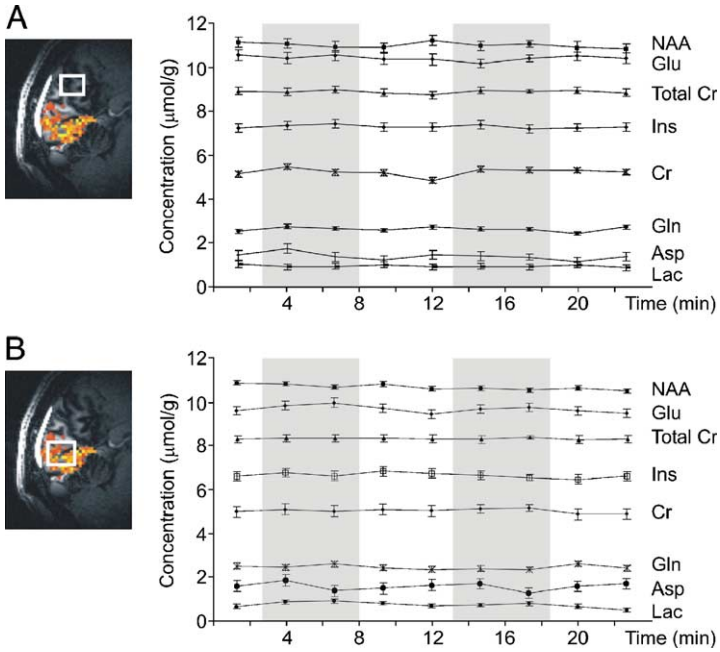

Fig. 2. Example of time courses of selected metabolites obtained by LCModel from the same subject during the functional paradigm (shaded areas correspond to visual stimulation), when the voxel was located outside (A) and inside (B) the visual cortex. Changes of metabolite concentrations during the functional paradigm were within ±0.2 μmol/g. Error bars indicated CRLB.

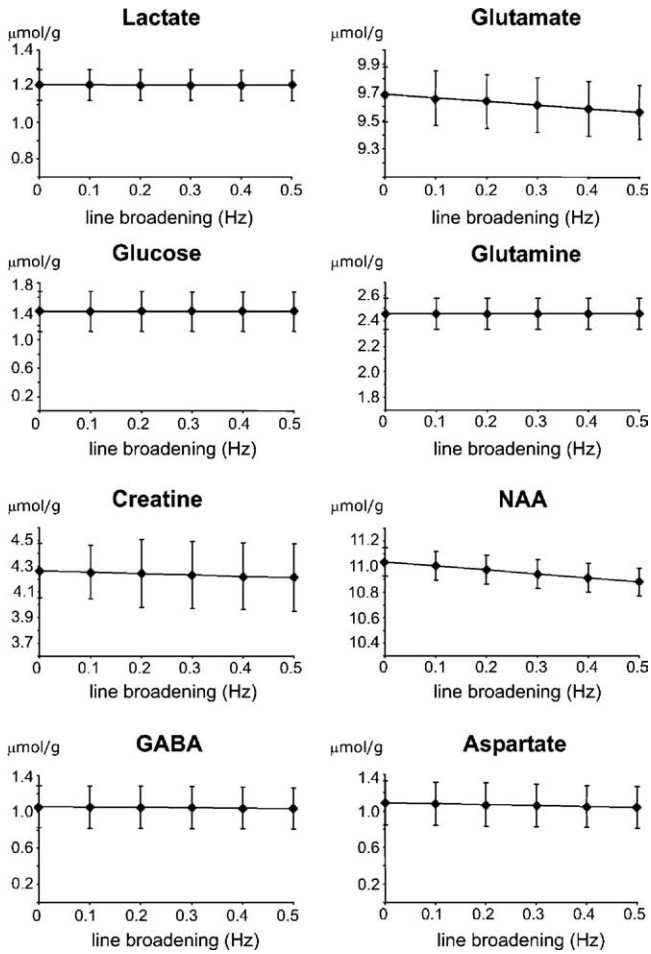

Fig. 3. Simulation of line-broadening effects on selected metabolite concentrations quantified by LCModel. Error bars correspond to CRLB. Estimates of metabolite concentrations were reproducible within the CRLB of the fit for a line broadening up to 0.5 Hz. A slight tendency in decreasing the estimated concentrations was observed for NAA, Cr and Glu. Lactate quantification was nearly unaffected by the tested line-width changes.

Lac (0.1%) were almost unaffected by the tested line-width changes.

4. Discussion

In the present study, a 7 T magnetic field was used to optimize the sensitivity of spectroscopy studies for functional applications. An obvious advantage of ultrahigh magnetic field is the increased SNR compared to lower fields, thus making possible to improve the design of functional protocols in terms of study duration, eventually with a temporal resolution of few seconds when using event-related paradigms. Most importantly, the increased sensitivity at 7 T potentially allows investigating the time course of metabolites even on single subjects.

A high number of metabolites was investigated at ultrashort TE, which minimized T_2 weighting and J modulation. Moreover, in these experimental conditions, the measured signal gave information about the *concentration* of metabolites in the voxel of interest, even if single-voxel ^{1}H-MRS cannot reach the suitable spatial resolution to differentiate between cellular compartments, just like any other modern noninvasive imaging modality.

The reproducibility of the spectra between different sessions, especially in the region at 1.5 ppm (Fig. 1), demonstrated high performance of localization. Subcutaneous lipids from outside the VOI can in fact contaminate the region at 1.5 ppm with broad signals, whose phase can depend on the distance between the VOI and the lipid-containing tissue [21]. The flat residual obtained when subtracting spectra (32 scans average) during stimulation and rest conditions (Fig. 1) verified a high stability of the system and reproducibility of the measures within each experimental session. The absence of large peaks in the difference spectra suggested that possible concentration changes during activation were within the noise level. The previously reported increases in lactate of 50% and higher [1,2,5], corresponding to almost 0.5 μmol/g, were not present in the difference spectra when the voxel was located inside the visual cortex (Fig. 1B).

The sensitivity threshold of single-subject studies can in general be expressed by the CRLBs, provided that these are smaller than the intrinsic variations of the measurement performed in control conditions (voxel outside the visual cortex). CRLBs are indeed an estimate of the precision of metabolite concentrations quantified by LCModel. The sensitivity threshold of our experiment was around 0.1 μmol/g for lactate and 0.2 μmol/g for most of the other quantified metabolites (Table 1). The analysis of the time courses of metabolites obtained when the voxel was located inside the visual cortex indicated that variations of all metabolites were small, within ±0.2 μmol/g (Fig. 2B). Higher S.D. of lactate compared to CRLB (Table 1) suggested the possibility of detecting lactate concentration changes during the functional paradigm in single subjects.

A previous study has reported that the BOLD effect, due to decreased susceptibility effects resulting from the local hyper-oxygenation of blood, can alter the T2* of both water and metabolite signals, introducing a line narrowing of the spectrum during activation [15]. The observed line narrowing was small (around 0.2–0.3 Hz at 4 T) and discernible only on the strongest singlets of the spectrum. Differentiating line width from concentration effects is generally not obvious, but it is still feasible by examining difference spectra. In fact, subtraction of two peaks with same frequency, same integral intensity and different line widths results in a small narrow peak in the difference spectrum, approximately two times narrower than the characteristic line width. Any change ascribed to an altered concentration should instead appear in the difference spectrum as a peak with the same intrinsic line width. Narrow small peaks, due to the BOLD effect, were observed in the difference spectra of our study at 2.0 and 3.0 ppm (Fig. 1B).

In theory, the time courses obtained by deconvolution algorithms such as the LCModel used here should not be influenced in a major way by the line narrowing

introduced by the BOLD effect. Yet, even if LCModel is to a certain degree able to take into account line-width changes, small effects on concentration determination cannot be a priori excluded. Our simulations suggested that possible functional concentration changes estimated by LCModel in the order of 1% may be affected by line-width variations that have not been accounted for. Such effect can be assessed by analyzing the presence of real concentration effects in the difference between spectra obtained at rest and during activation with applied appropriate line broadening in order to deal with line-width changes. The estimated concentration of lactate was nearly unaffected by line broadening up to 0.5 Hz, thus ensuring that lactate changes revealed by LCModel are a robust estimation of concentration effects.

5. Conclusion

We conclude that with the present experimental conditions functional concentration changes bigger than 0.2 μmol/g should be detectable at 7 T in single subjects for most metabolites. Our data also suggested that during prolonged visual stimuli these changes are within ±0.2 μmol/g. Furthermore, we conclude that minute concentration changes on the order of a few percentage may be affected by the BOLD line-narrowing effect.

Acknowledgments

This study was supported by grants NIH P41RR08079 and R01NS38672, the Keck Foundation and Mind Institute.

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