Diffusion-weighted spectroscopy allows to probe ^{13}C labeling of glutamate inside distinct metabolic compartments in the monkey brain

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Introduction

Diffusion-weighted (DW) NMR spectroscopy is generally accepted to be a valuable tool to probe cell compartmentation *in vivo* [1]. Although there is little doubt on the actual existence of compartments with different diffusion properties in the brain, they are not clearly identified, and the extent to which DW-spectroscopy effectively allows to probe them *in vivo* remains to be explored experimentally. Since the various diffusion compartments certainly closely match various anatomical/functional compartments, it is legitimate to expect some *metabolic* difference associated with these various *diffusion* compartments. Combining oxidative metabolism measurement during a ^{13}C-labeled glucose infusion with DW-spectroscopy could therefore provide direct evidence that some compartments with different apparent diffusion coefficients (ADC) are labeled at different rates. In this context, the aim of the present work was to find out whether the rate of glutamate ^{13}C labeling in the monkey brain depends on diffusion weighting.

Materials and Methods

NMR acquisition The study was conducted on 2 male macaque monkeys, with a total of 5 experiments. Experiments were performed on a whole-body Bruker 3 T magnet equipped with a gradient coil reaching 44 mT/m in 400 µs. The monkey head was positioned in a stereotaxic frame, the body being in the Sphinx position. Femoral veins were canulated for blood sampling, [U-$^{13}C_6$] glucose infusion and intravenous anesthesia (propofol) [2]. RF emission and reception was achieved by a circular surface coil (4.5 cm diameter). Signal was localized in an 18×18×18 mm^3 voxel (5.8 mL) (fig. 1) with a ~1:1 ratio of gray matter (GM) and white matter (WM). A DW-STEAM (TE/TM=21/110 ms) sequence optimized for glutamate detection was used [3]. Baseline spectra were acquired at b~0 and b~3000 s/mm^2 before the infusion (NT=512 for each b value). During the 2-hour ^{13}C glucose infusion, scans were acquired at b~0 and b~3000 s/mm^2 in an interleaved manner. Measurement of glutamate enrichment was based on the method proposed in [2]. Basically, the subtraction of ^1H spectra collected during the ^{13}C infusion from the baseline ^1H spectrum results in difference spectra where only ^{13}C-labeled metabolites remain.

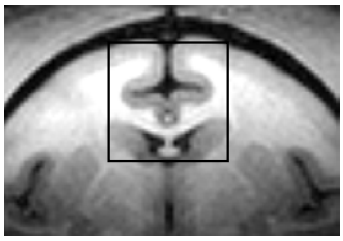

Fig. 1: *Position of the 5.8 mL voxel in the monkey brain.*

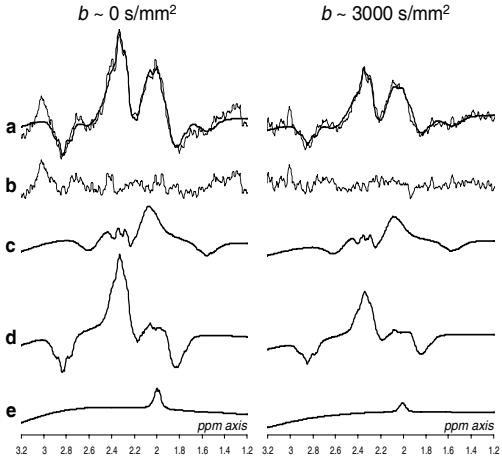

Fig. 2*. Typical difference spectra collected in one experiment 110 to 120 min after glucose infusion was started, for b~0 (left) and 3000 s/mm^2 (right). a) Data and LCModel fit. b) Residuals. c) Glutamate enriched in position C3. d) Glutamate enriched in position C4. e) Lorentzian lineshape accounting for NAA enriched in position C6 and potential subtraction error.*

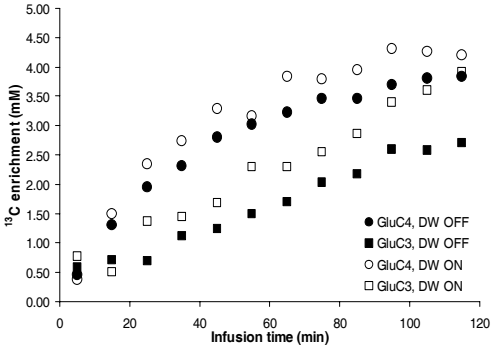

Fig. 3*. ^{13}C enrichments mean time-courses (5 experiments) measured for glutamate C3 (square) and C4 (circle), at b~0 (filled symbols) and b~3000 s/mm^2 (opened symbols).*

Spectra processing The scans collected in an interleaved manner during the infusion were reorganized in two separate data-sets, one corresponding to b~0 and the other to b~3000 s/mm^2. Scans were summed in blocks of 128 scans (10 minutes resolution). Blocks were subtracted from the baseline spectrum, yielding difference spectra which were analyzed by LCModel, using a simulated basis-set consisting in simulated model spectra for glutamate labeled in position C3 and labeled in position C4 (plus a Lorentzian peak resonating at 2.01 ppm in order to take into account the slow enrichment of NAA or possible subtraction error of the NAA peak). The time-courses were finally summed over the five different experiments. Glutamate enrichment time-courses at b~0 and b~3000 s/mm^2 were divided by the corresponding baseline glutamate signal to yield fractional enrichments.

Results

Typical difference spectra obtained at the end of glucose infusion for b~0 and 3000 s/mm^2 and the corresponding LCModel fits are presented on figure 2. The average ^{13}C glutamate time-courses (after conversion to mM based on a total glutamate concentration of 10.8 mM [2]) are presented on figure 3. It appears that ^{13}C labeling occurs at a higher rate at higher b value, slightly but significantly (p<0.0001, paired t-test on the 12 time-points) for glutamate C4 and more markedly for glutamate C3 (p<0.0001). As a matter of fact, the C3 time-course looks much closer to the C4 time-course at b~3000 s/mm^2.

Discussion

Existence of different diffusion compartments for glutamate If glutamate ADC was homogeneous in all cellular compartments, diffusion-weighting would not affect the relative contribution of these compartments to the total signal (the contribution of each compartment would simply be scaled by the factor exp(-b.ADC)). Therefore, the dramatic dependence of ^{13}C-glutamate time courses on diffusion-weighing demonstrates that different diffusion compartments exist for glutamate in the monkey brain, and that these compartments are associated with specific metabolic compartments.

Separation of gray and white matter metabolism using DW-spectroscopy? Based on the comparison of glutamate ADC in the monkey brain and in the rat brain, we have recently hypothesized [3] that glutamate ADC may be higher in white matter (WM) as compared to gray matter (GM). This trend was confirmed in the human brain [4], at least for NAA, creatine and choline (glutamate ADC was not measured). This idea is qualitatively consistent with the observed time-courses: at b~3000 s/mm^2, the contribution of high-metabolism [5,6,7], slow-diffusing GM would become predominant compared to the contribution of low-metabolism [5,6,7], fast-diffusing WM. A preliminary analysis (not detailed here) of the measured time-courses at b~0 and 3000 s/mm^2 using a two-compartment metabolic model where metabolic flux and glutamate ADC in GM and WM are simultaneously fitted yields values that are consistent with the current knowledge of brain metabolism and diffusion. This suggests that (*i*) the effect of diffusion-weighting on ^{13}C-glutamate time-courses mostly originates from GM/WM compartmentation, and that (*ii*) diffusion-weighting may allow to quantitatively separate GM and WM metabolism.

Conclusion

^{13}C-glutamate enrichment as measured during a ^{13}C-glucose infusion in the monkey brain proves to occur at a faster rate when diffusion-weighting is applied. This work demonstrates the existence of different glutamate diffusion compartments (possibly gray and white matter) associated with different metabolic rates. This potentially opens a new way to separate metabolic compartments when measuring the TCA cycle flux by NMR spectroscopy.

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