Neuronal and astrocytic metabolites exhibit different diffusion behavior, as observed by diffusion-weighted spectroscopy at ultra-long diffusion times.

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Target audience

This work should be of interest for researchers investigating how diffusion-weighted NMR spectroscopy may be used to characterize brain cell geometry *in vivo*.

Purpose

Diffusion-weighted (DW) NMR spectroscopy offers a unique insight into the intracellular space *in vivo* [1]. The apparent diffusion coefficient (ADC) is related to the average quadratic displacement $\langle x^2 \rangle$ during the diffusion time t_d. *In vivo*, cell membranes and subcellular structures hinder the translational displacement of metabolites. If diffusion time is long enough, the cell geometry is expected to have a significant impact on the ADC [2]. In a previous study carried out in the monkey brain with t_d varying up to 1 s, we observed that metabolites ADC barely depends on t_d suggesting that metabolites diffusion is typical of free diffusion as might occur along long fibers such as axons, dendrites and astrocytic processes [3]. Indeed, in this former study, a model describing diffusion within infinitely long fibers could be used to fit the data. However, real fibers are supposed to extend over a finite length, resulting in some restriction at fibers extremity. Therefore, in an attempt to provide a better characterization of the cellular architecture, we performed new experiments in the monkey brain and investigated metabolites diffusion over a larger time window with t_d varying up to 2 s. The ADC of five brain metabolites diffusing in different compartments (tNAA and Glu in neurons, tCho and Ins in astrocytes and tCr in both neurons and astrocytes) were measured and analyzed. Data were fitted with a model of diffusion within spheres, whose diameters are considered to somehow represent cell dimensions (i.e. fiber length).

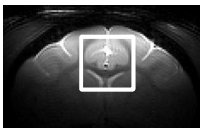

Fig.1: Position of the 5.8 mL voxel in the monkey brain on a T2-weighted image*

Materials and Methods

Experiments: Experiments were performed on a Varian/Agilent 7 T primate system (gradient coil reaching 100 mT/m along each axis in 325 µs). A ^{1}H quadrature surface coil was used for RF transmission and reception. A 5.8 mL voxel was positioned in the monkey brain (**Fig. 1**). Shimming was performed with Fastmap. Water and metabolites DW spectra were acquired using a modified STEAM sequence (TR/TE=2800/18 ms), for b=0 and 3000 s/mm^2, at different t_d reached by varying mixing time TM. Since at long t_d cross-terms between diffusion gradients and other gradients become significant and lead to biased ADC, additional DW-spectra were acquired with diffusion gradients of opposite polarity [3, 4]. Experiments were carried out on healthy anesthetized macaque monkeys. Spectra were acquired for t_d=82, 357, 507, 657, 1007 and 2007 ms and with an increased number of averages to keep similar SNR for each t_d. Four or five different t_d could generally be acquired during each session. Since at TE=18 ms, the macromolecules and lipids contaminate the brain metabolite signal, a macromolecule spectrum was acquired for each experiment and each t_d (except for t_d=1007 and 2007 ms as macromolecules and lipids signal has vanished) using an inversion recovery preparation (TI=590 ms).

Processing: Scan-to-scan phasing, eddy current correction and macromolecules subtraction were performed. Spectra were analyzed with LCModel [5] with a different basis-set for each TM. The ADC of five metabolites was quantified: total NAA (tNAA), total creatine (tCr), total choline (tCho), glutamate (Glu) and myo-inositol (Ins).

Modeling: A model describing diffusion within cells was implemented on Matlab using Balinov formalism [6]. In this model, cells were modeled by spheres of diameter d and the ADC decreases from D_{intra} towards 0 at long t_d (with D_{intra} the intracellular diffusion coefficient accounting for potential intracellular tortuosity over short time scales). Fit was performed on the ADC of each brain metabolite. Monte Carlo simulations were also performed. Briefly, the standard deviation of the experimental data was estimated from the difference between the best fit and the experimental data. Random noise was generated with the same standard deviation, and added to the best fit to generate a new dataset, which could be analyzed using the model. This procedure was repeated a thousand times for each brain metabolite. Statistics and probability distribution of d could then be estimated.

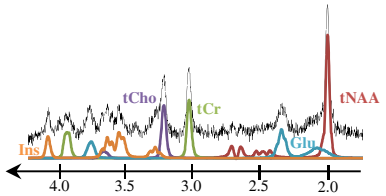

Fig. 2: Example of in vivo spectra acquired at b=3000 s/mm² with t_d=2007 ms, and LCModel analysis.

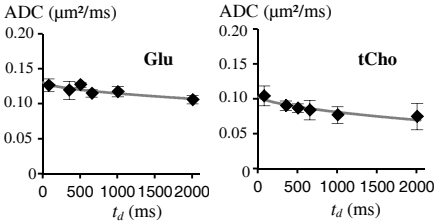

Fig. 3: Metabolites ADC were measured for six t_d. Glu and tCho experimental data (⧫) and best fit are represented. N=5 experiments were averaged at t_d=82, 357 and 1007 ms, N=4 at t_d=507 and 657ms, N=3 at t_d=2007 ms.

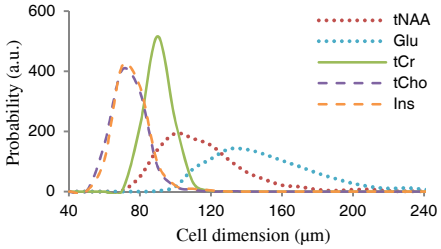

Fig. 4: Histogram of Monte Carlo simulation results. The plots show the probability distribution of the cell dimension for the five brain metabolites.

Results and Discussion

Metabolites diffusion in vivo: Good quality spectra were obtained for all t_d, as exemplified in **Fig. 2** for t_d=2007 ms. Glu and tCho ADC as a function of t_d, averaged over the different experiments, are displayed in **Fig. 3**. Over this large time-window, a slight decrease of ADC at longer t_d could be observed. Metabolite ADC averaged over all experiments and all t_d were: ADC_{tNAA}=0.112±0.010 µm²/ms, ADC_{tCr}=0.114±0.012 µm²/ms, ADC_{tCho}=0.086±0.011 µm²/ms, ADC_{Glu}=0.118±0.008 µm²/ms and ADC_{Ins}=0.092±0.012 µm²/ms.

Diffusion modeling: The model yielded a good fit of the experimental ADC. Best fit values obtained for d were: d_{Glu}=150 µm, d_{tNAA}=116 µm, d_{tCr}=96 µm, d_{Ins}=79 µm and d_{tCho}=78 µm. The extreme difference in tCho and Glu cell dimensions might conceal a difference between metabolites diffusing in astrocytes and in neurons. When ordering metabolites from smallest to highest d, we get tCho<Ins<tCr<tNAA<Glu. This is consistent with the idea of tCho and Ins diffusing in astrocytes, whose processes are shorter than axons and dendrites in which neuronal tNAA and Glu are diffusing. The fact that tCr lies in between is also consistent with the idea that tCr shows no preferential cell compartmentation.

Monte Carlo simulation: The probability distributions of the cells dimensions are shown on **Fig. 4**. tCho and Ins distributions completely overlap, which is very consistent with the fact that they are both preferentially compartmentalized in the astrocytes. No overlaps can be observed between tCho and Ins distribution on one side, and Glu distribution on the other side, supporting the idea that Glu diffuses in a distinct compartment (neurons). tNAA and Glu distributions largely overlap, suggesting that they share some common neuronal compartmentalization. tCr distribution overlaps with all metabolites distributions which is consistent with the idea that tCr diffuses in all cells. The overlap between the tNAA and tCho and Ins distributions might reflect measurement uncertainty. Finally, the fact that tCho and Ins compartment size distribution is much narrower than tNAA and Glu distributions suggests that the size of astrocytes might be much more homogeneous than the size of neurons (some having much longer axons and dendrites than others).

Conclusion

In this study, brain metabolite ADC was measured in the monkey brain at ultra-long diffusion times (up to 2 s). Metabolites ADC exhibit a slight decrease with t_d, and modeling suggests that metabolites exhibit different behavior depending on their cellular compartmentation in neurons and astrocytes. Therefore, DW-spectroscopy at ultra-long t_d might allow probing neurons and astrocytes spatial extensions. The model applied here corresponds to a very simplified geometry. A more realistic model consisting in spheres (cell bodies) prolonged by fibers of finite length would certainly allow a better characterization of metabolite-specific behaviors, and a more accurate determination of cell geometrical parameters. To the best of our knowledge, no such analytical model currently exists, but we are going to investigate how numerical models may be used to analyze our data.

[1] Nicolay *NMR in Biomed* 2001 [2] Tanner and Stejskal *J. Chem. Phys.* 1968 [3] Najac *ISMRM* [4] Neeman *MRM* 1991 [5] Provencher *MRM* 1993 [6] Balinov J. Magn. Reson. 1993