# Brain metabolites diffuse "freely" in white and grey matters: new insights into cellular architecture by diffusion-weighted spectroscopy in the Human brain.

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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

Due to the specific intracellular compartmentation of brain metabolites, diffusion-weighed (DW) $^1$H NMR spectroscopy is a powerful tool to investigate brain 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*, geometrical constraints due to cell membranes and subcellular structures hinder the translational displacement of metabolites. If diffusion time is long enough, restriction by cell walls is expected to have a significant impact on the ADC [2], with metabolite ADC decreasing towards an asymptotic value which depends on cell geometry. In long and thin fibers isotropically distributed, after an initial fast decrease from $D_{intra}$ to $D_{intra}/3$, metabolite ADC should remain stable around $D_{intra}/3$ (with $D_{intra}$ the intracellular diffusion coefficient). In contrast, it should decrease towards 0 in closed cell bodies. One of our previous study, carried out with $t_d$ varying from 82 ms to 1 s in the monkey brain, revealed that brain metabolite ADC barely depends on $t_d$, meaning that observed metabolites diffusion essentially reflects unrestricted diffusion, such as occurring in long and thin fibers (axons, dendrites, glial processes…) [3]. Nevertheless, spectra were acquired in a voxel containing equal proportions of white and grey matters, resulting in no tissue specificity. In order to assess potential differences in the dependence of metabolite ADC in both tissues, we decided to explore metabolites diffusion in voxels with various proportions of white matter (WM) and grey matter (GM) in the Human brain, for $t_d$ varying from 100 ms to 720 ms.

## Materials and Methods

***Experiments:*** Experiments were performed on a Philips Achieva 7 T MRI scanner (gradient coil reaching 33 mT/m along each axis). A head RF coil (quadrature transmit and 32-channel receive) was used for all measurements. Data were collected from 7 healthy volunteers (age=23±1 years). Two sets of experiments were carried out, either in a 6 mL voxel positioned in parietal white matter, or in a 6 mL voxel positioned in occipital grey matter as shown in **Fig. 1**. Voxels-of-interest (VOI) were selected using a 3D-$T_1$-weighted image. Shimming was performed using a pencil beam method employing second-order shims. Water and metabolites spectra were acquired using a modified DW-STEAM scheme (TE=50 ms, TR=2-3 cardiac cycles (triggering using PPU), pulse duration 24 ms), for $b$=0 and 3000 s/mm², at different $t_d$ reached by varying mixing time (TM=80, 530 and 700 ms). To get rid of tissue anisotropy, the trace of the diffusion tensor was actually acquired, with measurements performed along the three orthogonal directions yielding maximal gradient amplitude ([1 1 -0.5], [-0.5 1 1] and [1 -0.5 1]) [4]. Since at long $t_d$ cross-terms between diffusion gradients and other gradients may lead to biased ADC, additional DW-spectra were acquired with diffusion gradients of opposite polarity for each direction and each $t_d$ [5]. Spectra were acquired with an increased number of averages to keep similar SNR for each $t_d$.

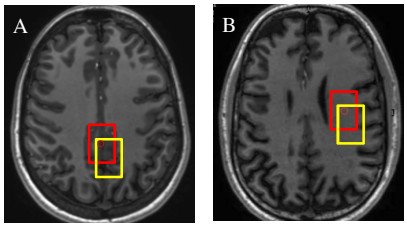


*Fig. 1 Position of a 6 mL voxel in occipital grey matter (A) and parietal white matter (B) on 3D-T1-weighted image. Voxels locations are shown for NAA (red box) and water (yellow box)*

***Processing:*** Scan-to-scan phasing, frequency drift and eddy current corrections were performed. Spectra were analyzed with LCModel [6] with a different basis-set for each TM. At $b$=3000 s/mm², the geometric mean of the signal measured with both gradients polarities was calculated. The ADC was finally quantified for three brain metabolites: total NAA (tNAA), total creatine (tCr) and choline compounds (tCho). 3D-$T_1$-weighted images were segmented using an in-house routine to determine the proportions of GM, WM and cerebrospinal fluid (CSF) in the VOI.

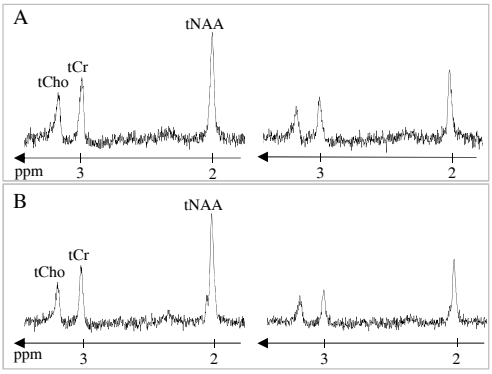


*Fig. 2 Examples of in vivo spectra acquired at b=0 (left) and 3000 s/mm² (right) in one direction for $t_d$=720 ms in GM (A) and WM (B)*

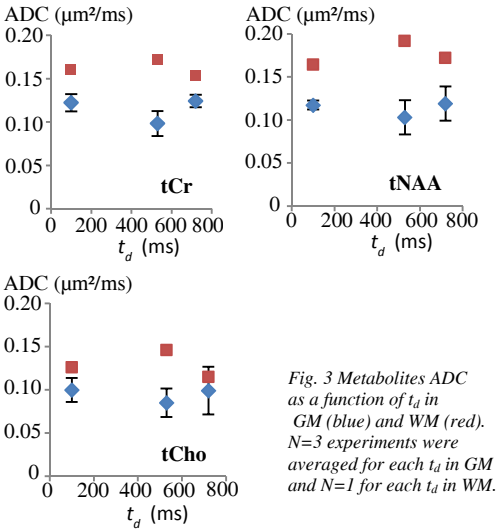


*Fig. 3 Metabolites ADC as a function of $t_d$ in GM (blue) and WM (red). N=3 experiments were averaged for each $t_d$ in GM and N=1 for each $t_d$ in WM.*

## Results

Parietal voxels predominantly reflected WM (85±7% of WM, 14±7% of GM and 2±1% of CSF), whereas occipital voxels predominantly reflected GM (23±6% of WM, 66±10% of GM and 11±1% of CSF). Good quality spectra could be obtained for all $t_d$ and $b$ values, as exemplified on **Fig. 2** for $t_d$=720 ms. As already reported [7], metabolite ADC was lower in GM ($ADC_{tNAA}$=0.108±0.005 µm²/ms, $ADC_{tCr}$=0.111±0.011 µm²/ms and $ADC_{tCho}$=0.092±0.007 µm²/ms, mean and s.d calculated over all subjects and all $t_d$) compared to WM ($ADC_{tNAA}$=0.176±0.014 µm²/ms, $ADC_{tCr}$=0.162±0.009 µm²/ms and $ADC_{tCho}$=0.129±0.016 µm²/ms). No time-dependence of metabolite ADC could be observed, either in WM or in GM (**Fig. 3**), meaning that observed diffusion is largely unrestricted for these time-scales.

## Discussion and Conclusion

The specific compartmentation of metabolites (tNAA in neurons, tCho mainly in astrocytes, and tCr in all cells) opens a unique insight into the different cellular architectures in WM and GM Here, no dependence of the ADC on $t_d$ was observed, neither in predominantly WM, nor in predominantly GM voxels. This stability suggests that the major fraction of brain metabolites diffuses in long fibers rather than in cells bodies. For WM, it is relatively well established that neurons are essentially characterized by long, myelinated axons. Even astrocytes in WM have an elongated form. In this context, the stability of metabolites ADC in WM is largely confirmatory of this fiber-like cell structure. In contrast, the fiber-like stability of metabolite ADC in GM goes against the common intuition of neuronal soma occupying a significant volume fraction. Some additional experiments would be required to perform data modeling and derive values about soma diameters and the relative volume fractions between soma and fibers. This study opens new perspectives for interpreting diffusion measurements in the Human brain. The absence of significant restriction suggests that metabolite ADC may be very sensitive to $D_{intra}$, i.e. to intracellular viscosity, molecular crowding and short distance obstacles (organelle content and intracellular tortuosity), even at relatively long $t_d$ characteristic of clinical scanners. In contrast, variations in cell size may be more difficult to detect within the measurement time-scales of DW-spectroscopy achievable on clinical scanners.

[1] Nicolay *NMR in Biomed* 2001 [2] Tanner and Stejskal *J. Chem. Phys.* 1968 [3] Najac *ISMRM* 2012 [4] Marchadour *ISMRM* 2012 [5] Neeman *MRM* 1991 [6] Provencher *MRM* 1993 [7] Kan *MRM* 2012.