Localized double-Pulsed-Field-Gradient MRS of CNS metabolites

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Introduction. Conventional single-PFG MR approaches such as Diffusion-Tensor-Imaging (DTI)[1] are widely used to study the macroscopic organization of coherently organized systems such as CNS White Matter (WM). By contrast, detection of salient underlying topological features in systems characterized by a high degree of disorder – such as in the CNS Gray Matter (GM) – remains highly elusive. Angular double-Pulsed-Field-Gradient (d-PFG) diffusion MR[2-5] has gained increasing attention in recent years due to its unique ability to deal with this problem of randomly-oriented systems: d-PFG can detect microscopic anisotropy[3] (μA) and a compartment's shape anisotropy[4] (csA), that can be related to the restricting length scale and compartment eccentricity, respectively[5]. So far, all d-PFG MR measurements in biological systems have been conducted on the water signals, which being ubiquitous lack any compartmental specificity. Applying d-PFG MR to the resonances of endogenous metabolites could therefore be highly valuable, since (A) metabolites may reside in known specific cellular compartments, thereby offering a means to increase the compartmental specificity of the measurement[6,7], and (B) metabolic μA and csA properties have never been previously explored. We describe here a new form of localized d-PFG MRS based on the spectrally-selective excitation of resonances of interest. This sequence revealed, for the first time, μA and csA characteristics of resolved metabolic species –offering a powerful means to studying microstructural features in tissues.

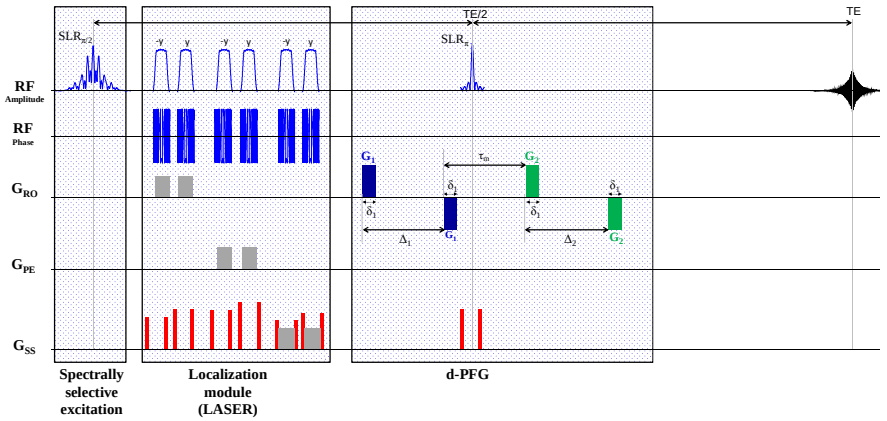

Fig. 1. Localized d-PFG MRS sequence. Preparation, localization, and d-PFG blocks are indicated by shaded regions. Slice selection and crusher gradients are depicted in gray and red, respectively.

Methods. Localized d-PFG MRS experiments (Figure 1) were performed on fresh pig spinal cords and on mouse brains (placed in a 10mm NMR tube and immersed in Fluorinert) on a 9.4T Bruker Avance magnet with a micro5 imaging probe capable of producing PFGs up to 300 G/cm in all directions. In the mouse brain, a 2x2x5mm^3 voxel was located in the striatal GM; for the pig spinal cord, two voxels were selected for localized d-PFG MRS focused on the GM (0.8x1.7x5mm^3) and the WM (2x2x5mm^3), respectively. The sequence employed spectrally selective Shinnar-Le Roux[8] (SLR)-derived multiband pulses (τ_p=32ms) to excite the resonances of interest (in this case Cre, Cho, NAA and Lac). Importantly, the pulse was designed such that <1E-4 of the water signal is excited. Spatial localization was achieved by LASER modules[9]: three successive blocks comprising two slice-selective adiabatic π pulses with bandwidth of 10 kHz (to minimize chemical shift displacement errors), a 5 ms duration, which were phase cycled through {y,-y} alternations. An SLR-generated spectrally-selective π pulse (BW=4kHz, offset=-2480Hz from the on-resonance water) was used to refocus the phase evolution during the excitation, leading to an echo at t=TE but without exciting/inverting the water signal. The d-PFG block consisted of two bipolar pairs of PFGs, separated by a mixing time (τ_m) (dark blue and green G's, respectively, in Fig. 1). The d-PFG MR parameters were δ_1= δ_2=2ms, Δ_1= Δ_2=40ms and $|\mathbf{G_1}|=|\mathbf{G_2}|$=57.6 G/cm. Angular d-PFG MRS[2-5] experiments were performed in the X-Y plane with τ_m=0ms or with τ_m=40m, seven ψ-values (ψ =0°,45°…360°), NA=160, leading to a total experiment time of ~1.5h. The data are shown normalized to S(ψ =0°). No water suppression was used.

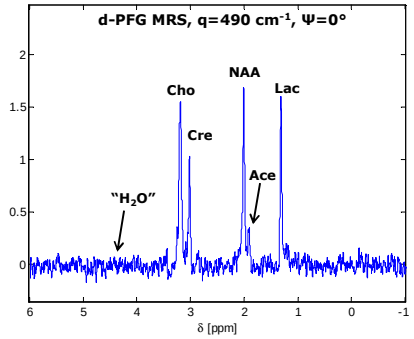

Fig. 2. Representative d-PFG MRS data.

Results and Discussion. Figure 2 shows a representative spectrum for the d-PFG weighted signal (S(ψ=0°) with a long mixing time) from the striatal GM of the mouse brain. Notice the spectrum's high-quality even at q=490cm^{-1}, reflecting the fact that only the resonances of interest were excited. Figure 3a shows the locations of voxels targeted in a pig spinal cord (from which a reference T2 weighted image was subtracted). Figure 3b shows the angular signal dependence for the long-τ_m regime in the GM voxel. A clear cos(2ψ) behavior is observed for all metabolites, evidencing that the metabolites are diffusing in randomly oriented compartments characterized by a finite compartment shape anisotropy (i.e. the underlying topology of the compartments is of an eccentric shape). For the WM voxels the oscillation's amplitude was much smaller than for the GM, suggesting a much less eccentric diffusion environment for its metabolites (Fig. 3c). Localized d-PFG MRS with zero mixing times revealed a bell-shaped profile in the WM with an increase of ~20-40% at ψ=180°for all metabolites; while in the GM, the τ_m=0ms experiments showed a modulated profile[5] with an increase of more than 250% at ψ=180° (not shown). These results clearly indicate the presence of μA for metabolites in both GM and WM, where the restricting length scale in WM is clearly smaller for the metabolites compared to GM. The presence of csA, as manifested by cos(2ψ) angular signal dependence (Fig. 3), was also clearly evidenced by all metabolites when long- τ_m experiments were run in the striatal GM of the *ex-vivo* mouse brain (not shown).

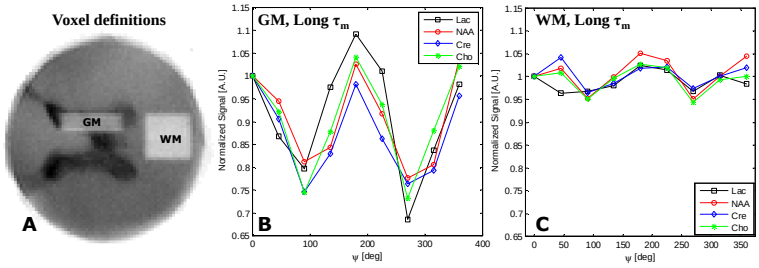

Fig. 3. Localized d-PFG MRS results. **(A)** Targeted voxels. **(B,C)**, normalized angular dependence for long τ_m experiments in the GM and WM, respectively. Note the pronounced modulation observed due to csA.

Conclusions. A robust localized d-PFG MRS sequence was developed and used to unravel microstructural features of tissues, as viewed from the perspective of specific metabolites. The presence of μA and csA was demonstrated for the first time in both WM and GM tissues for Cho, Cre, NAA and Lac. This methodology opens the possibility to utilize the chemical shift dimension for studying the μA and csA properties (and hence underlying compartmental dimensions and eccentricities) in both normal and diseased tissues. We expect that insight into the underlying microstructure and function in tissues (especially in GM) could be obtained due to the specificity of some of the metabolites to cellular compartments.

References. [1] Basser PJ et al., Biophys. J. **66 (**1994) 259. [2] Mitra PP, Phys. Rev. B, **51 (**1995) 15074. [3] Koch MA and Finsterbusch J, Magn. Reson. Med. **60** (2008) 90. [4] Özarslan E, J. Magn. Reson. **199** (2009) 56. [5] Shemesh N and Cohen Y, Magn. Reson. Med. **65 (**2011) 1216. [6] Cohen Y and Assaf Y, NMR Biomed. **15** (2002) 516. [7] Updahay J et al., Magn. Reson. Med. **58** (2007) 1045. [8] Pauly J et al., IEEE Trans. Med. Imaging. **10** (1991) 53. [9] Garwood M and DelaBarre L., J Magn Reson **153** (2001) 155.