Resolving Cellular Specific Microarchitectures Using Double Pulsed Field Gradient Weighted, Relaxation-Enhanced Magnetic Resonance Spectroscopy

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Target audience. Researchers and clinicians interested in brain microstructure and in Magnetic Resonance Spectroscopy (MRS).

Purpose. Depicting cellular specific microarchitectures *in vivo*.

Introduction. Cellular-specific microarchitectures can be altered by neurodegeneration and neuroplasticity [1]. Water-based MR approaches for characterizing such changes are handicapped by the ubiquitous presence of water in every cell type and every micron-scale substructure in the brain. By contrast, ^{1}H MRS that targets selectively compartmentalized metabolites could offer a glimpse into specific compartments *in vivo* and noninvasively [2]. Recently, a new development termed Relaxation-Enhanced MRS (RE-MRS) has been shown to offer the sensitivity required to probe metabolic microstructures directly with high fidelity [3]. Here, a RE-MRS methodology capable of depicting specific cellular microarchitectures *in vivo* is employed to target N-Acetylaspartate (NAA) and myo-Inositol (mI) resonances specifically compartmentalized in neurons [4] and astrocytes [5], respectively. This cell-type specific targeting was combined with a novel double-Pulsed-Field-Gradient (dPFG) filter [6], designed to mitigate cross-terms arising from internal, susceptibility-induced gradients. This strategy permits the characterization of randomly oriented cellular-specific microstructures. These techniques can be generalized and offer novel ways of characterizing diseased and healthy CNS *in vivo*.

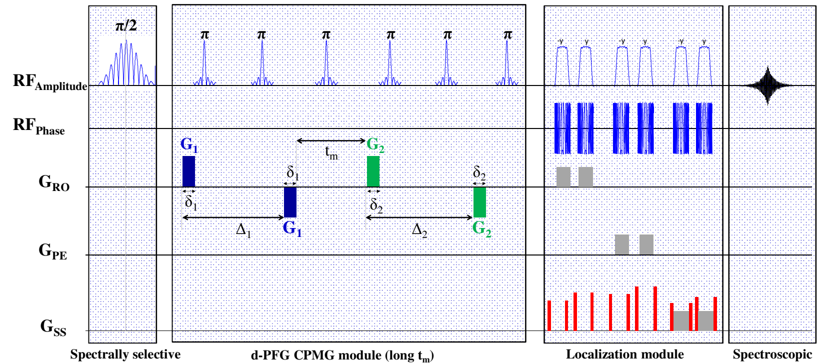

Fig 1. RE-MRS weighted by a dPFG filter and augmented by CPMG pulses that reduce the sequence's vulnerability to susceptibility-induced internal gradients.

Methods. All experiments in this study were conducted at the National High Magnetic Field Laboratory using the 21.1-T, 900-MHz ultra-widebore magnet equipped with a Bruker (Bruker-Biospin, Billerica, MA) Avance III console and a gradient system (RRI, Billerica, MA) capable of producing up to 60 G/cm in all directions. In this study, healthy juvenile Sprague-Dawley rats (N=6) weighing 220-300 g were examined. RE-MRS involves selectively exciting and refocusing only resonances of interest, which simplifies the spectra, avoids distortions, and enhances sensitivity [3]. The RE-MRS sequence used in this study is shown in Fig 1; it involved 8-ms excitation pulses focusing on two bands incorporating the NAA singlet resonance at 2.02 ppm and mI multiplet resonances at ~3.51 and ~3.61 ppm, followed by refocusing and LASER [7] pulses 4- and 5-ms long, respectively. The dPFG module of this sequence incorporates a CPMG block consisting of six refocusing pulses to alleviate potential artifacts caused by susceptibility-induced cross-terms. dPFG parameters were: $\delta_1=\delta_2=4.5$ ms, $\Delta_1=\Delta_2=64$ ms, $t_m=25$ ms $|\mathbf{G_1}|=|\mathbf{G_2}|=24$ G/cm, TR/TE=2500/167 ms, NA=160. The angular dPFG experiment was carried out by changing the relative angle between $\mathbf{G_1}$ and $\mathbf{G_2}$ between 0:45:360 degrees in the X-Y plane. Total scan time per trace was 6 m. Localization of a 5x5x5 (mm)^3 voxel centered in the rat brain was achieved via LASER pulses [7], and its accuracy was assessed by imaging the voxel prior to RE-MRS (Fig. 2, inset).

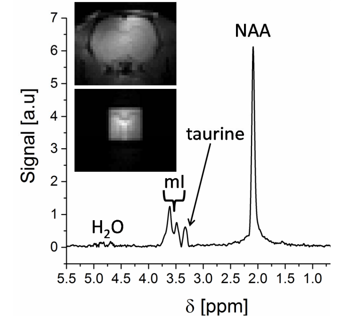

Fig 2. NAA, a neuronal marker, and mI, an astrocytic marker, are observed with excellent fidelity, a clean baseline and no water signal in this RE-MRS spectrum. Inset shows the quality of LASER localization.

Results and Discussion. One goals was to investigate whether the mI and NAA resonances could be resolved in the same spectrum, especially considering the relatively low intensity of the mI resonance and its proximity to the water peak. A representative RE-MRS spectrum (with dPFG gradient amplitudes set to 0) is shown in Fig 2. Clearly, mI and NAA are selectively excited, with no water signal residuals. Typical SNRs of >100 for the mI peak and >400 for the NAA resonance, were noted. A partial taurine resonance also appears in the spectrum because we have relaxed the pulse's pass-band conditions so as to better accommodate stop-bands near the water resonance. With this sensitivity, even challenging experiments such as dPFG MRS [3] can be performed on these cellular specific markers (Fig. 3). Representative spectra (Fig. 3, left panel) demonstrate the characteristic dPFG oscillation [8], whose origin can only be attributed to restricted diffusion within an ensemble of randomly oriented compartments. When neuronal vs. astrocytic oscillations are examined, good reproducibility is demonstrated (Fig. 3); naturally, the variance across animals is somewhat larger for mI, due to the *ca.* 1:4 SNR ratio of mI:NAA. Still, these similar curves unequivocally demonstrate the restricted nature of diffusion within each of these specific cell types. Quantifications of these signal modulations reveal similar microstructures for both cell types; this likely arises from the "filter" imposed by the selection of experimental parameters. Namely, the combination of metabolic diffusion coefficient, diffusion time, diffusion gradient duration, mixing time and gradient amplitude selectively probes–for our choice of parameters–the randomly oriented sub-compartments within these cells whose eccentricity is similar. A reasonable hypothesis is that in neurons, randomly oriented neuritis are highlighted whereas in astrocytes, the compartments probed are the randomly oriented astrocytic processes. Cell bodies and other larger and more isotropic components in each of these cell types seem to be filtered.

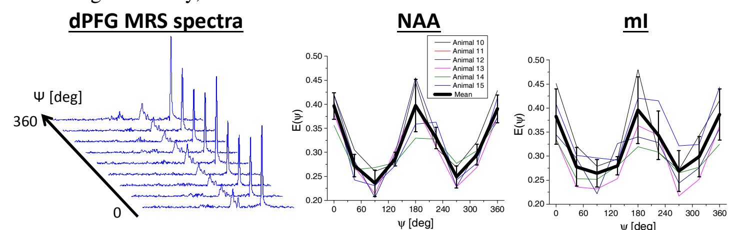

Fig 3. dPFG MRS spectra showing a clear cos(2ψ)-like modulations for both NAA and mI resonances, indicative of restricted diffusion in randomly oriented anisotropic compartments. Error bars reflect standard deviations.

Conclusions. We present the first demonstration of unequivocal restricted diffusion signatures of cellular-specific metabolites, and show that a subset of microstructures within neurons and astrocytes can be selectively probed. Our findings highlight the potential of this approach towards noninvasively probing cellular-specific microarchitecture modulations upon disease and in healthy tissues.

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