Diffusion Weighted Magnetic Resonance Spectroscopy of Peripheral Nerve at 18.8T

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Introduction – Diffusion anisotropy of water in neural tissue has been examined quite extensively, but compartment specific interpretation of diffusion characteristics is rather complex given that tissue water includes contributions from exchanging intra- and extracellular water. However, intracellular space can be probed selectively by measuring the diffusion characteristics of intracellular metabolites with diffusion weighted magnetic resonance spectroscopy (DW-MRS). To date, there have only been three studies that have measured anisotropic diffusion of intracellular metabolites in tissue, one measured N-acetyl Aspartate (NAA), creatine and phosphocreatine (tCr), and choline (Cho) diffusion in bovine optic nerve in-vitro (1), another measured NAA diffusion in human corpus callosum in-vivo (2), and more recently a paper measured the mean diffusivity (Trace/3 apparent diffusion coefficient (ADC)) and fractional anisotropy (FA) of NAA, tCr, Cho in the human brain in a variety of different regions (3). The former two studies, although they have measured anisotropy in the nerve and brain respectively, did not calculate the Trace/3 ADC or FA values for the metabolites. The latter study on the human brain was complicated by motion induced phase errors and large voxel sizes. The purpose of this study was to measure Trace/3 ADC and FA values of different metabolites in excised myelinated peripheral nerve of the frog at 18.8T.

Methods – Nerve sample preparation: All sciatic nerve samples were taken from adult *Xenopus laevis*, the African clawed frog. In total, ten nerve samples (5 frogs) were used in this study. Following euthanasia, ~3 cm segments of nerve were removed and placed in an oxygenated physiological buffer, and the perineurial sheath surrounding the nerve was removed. The nerve samples were immersed in Fluorinert and were aligned along the z-axis of the magnet by tying thread to one end and pulling the nerve (and Flourinert) into a small capillary tube with a 1 mm inner diameter, both ends subsequently sealed with parafilm. The capillary tube was then placed within a 5mm NMR tube along the z-axis and was surrounded by 99% D_2O (required for the lock signal). All nerve samples were placed in the NMR within 3 hours of extraction. NMR Experiments: Diffusion measurements were performed using an 800 MHz (18.8 T) Varian Inova NMR spectrometer running VNMRJ 1.1D, equipped with a XYZ-gradient HCN 5mm probe with a maximum gradient strength of ~30 G/cm along the X and Y axes and ~60 G/cm along the Z-axis, and a diffusion-weighted spin-echo pulse sequence with 5.0 ms diffusion gradient pulses (δ) and a diffusion gradient separation (Δ) of 20.0 ms. The echo time (TE) for the pulse sequence was 30 ms and the repetition time (TR) was 3 s. Water suppression was achieved using a water selective 90 degree pulse prior to the hard 90 in the spin-echo pulse sequence. The gradient strengths of the X, Y, and Z gradients were calibrated using a 600μL phantom containing a solution of NAA (3 mM), Cr (3 mM), and Cho (1 mM) and the known Cr diffusion coefficient of 0.80 x 10^{-3} mm^2/s (4). Seven separate calibrated b-values were measured for each direction (ranging from ~200 to 2300 s/mm^2) and the diffusion coefficients were calculated by measuring the slope of ln(signal intensity) vs b. The phantom ADC values in the X, Y, and Z direction were consistent with previously reported values (4) and were isotropic as expected. The nerve experiments were performed using the same b-values as in the phantom study. The number of averages used in the nerve experiments ranged from 64 to 512. The total scan time per nerve ranged from ~90 min to ~9 hr. The Trace/3 ADC and FA values of the metabolites were calculated assuming the X, Y, and Z diffusion coefficients were the eigenvalues of the diffusion tensor.

Results and Discussion – Example spectra, with 64 averages, in the parallel (Z) direction at low and high b-value are shown in Figure 1 and demonstrate the spectral quality of our data (average NAA SNR was 48). Figure 2 is an example plot of the signal intensity versus b-value for NAA and clearly highlights the greater diffusivity in the Z direction. The Trace/3 ADC and FA values for NAA, tCr, Cho, glutamate (Glu), and taurine (Tau) were determined (Table 1). The three main metabolites (NAA, tCr, and Cho) were found in all ten nerve samples, whereas the Glu and Tau, with much less SNR, were only seen in three and four samples, respectively. The Trace/3 ADC values range from 0.17 to 0.28 x 10^{-3} mm^2/s, which is consistent with previous work in human brain where the Trace/3 ADC ranges from 0.12 to 0.21 x 10^{-3} mm^2/s (5). However, the Trace/3 ADC of NAA, tCr, and Cho in the frog sciatic nerve followed the trend Cho < NAA < tCr which is contrary to the human brain where that trend was NAA < tCr ~ Cho (5). A recent paper reported a single direction ADC for Glu in the primate brain of 0.21 x 10^{-3} mm^2/s (6), which is consistent with our frog sciatic nerve Trace/3 ADC value for Glu of (0.17 ± 0.03) x 10^{-3} mm^2/s; the differences likely arising from the primate work measuring diffusion using a large voxel composed of both gray and white matter and in only one direction. The FA values for metabolites in the frog sciatic nerve ranged from 0.20 to 0.62, which are lower than FA values of water previously reported in the frog sciatic nerve of 0.73 (extrapolated assuming λ_1=parallel ADC and λ_2= λ_3=perpendicular ADC) (7). However, the FA values of NAA (0.45), tCr (0.62), and Cho (0.61) in the frog sciatic nerve are similar to white matter in the human brain, which ranged from 0.57 to 0.68 for the same three metabolites. tCr and Cho were found to have higher FA values in the frog sciatic nerve when compared with NAA. For tCr the parallel diffusion was significantly greater than NAA leading to this increase in FA, and for Cho the opposite was true where the perpendicular diffusion was significantly less than NAA causing the higher FA. NAA is located solely in the axons in the peripheral nerve and therefore it was expected to have a larger degree of anisotropy than tCr and Cho, since tCr and Cho are found in other structures such as Schwann cells which were thought to be more isotropic, and therefore the larger FA for tCr and Cho seems to be counterintuitive. However, this trend is consistent with studies in the human brain where tCr and Cho have higher mean FA values than NAA in the white matter. The Glu FA of 0.20 ± 0.06 was very low in comparison to the other metabolites, which may be due to the Glu being within vesicles (an isotropic environment) inside the axons, as well as the Schwann cells.

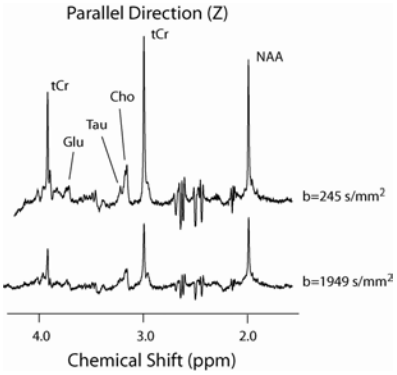

Figure 1 – Example spectra taken from low and high b-values in the Z-direction in the frog sciatic nerve.

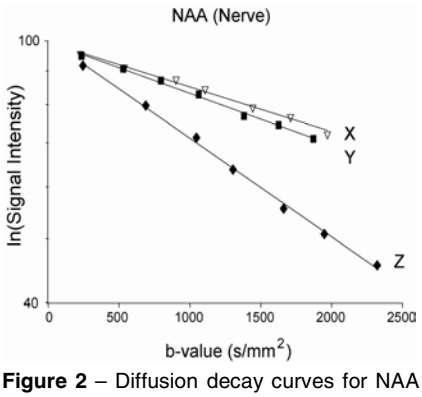

Figure 2 – Diffusion decay curves for NAA in the X, Y and Z directions in the frog sciatic nerve.

Table 1 – Trace/3 ADC and Fractional Anisotropy values of metabolites in the frog sciatic nerve (mean ± SD)

Metabolite	Trace/3 ADC (x10^{-3} mm^2/s)	Fractional Anisotropy
NAA (N=10)	0.22 ± 0.02	0.45 ± 0.13
tCr (N=10)	0.28 ± 0.03	0.62 ± 0.11
Cho (N=10)	0.17 ± 0.03	0.61 ± 0.11
Glu (N=3)	0.17 ± 0.03	0.20 ± 0.06
Tau (N=4)	0.25 ± 0.03	0.60 ± 0.10

References : (1) Assaf and Cohen. NMR Biomed 1999; 12:335-344, (2) Kroenke et al. MRM 2004; 52:1052-1059, (3) Ellegood et al. MRM 2006, *in press*. (4) Nicolay et al. NMR Biomed 1995; 8:365-374. (5) Ellegood et al. MRM 2005; 53:1025-1032. (6) Valette et al. NMR Biomed 2005, *online*. (7) Beaulieu and Allen MRM 1996; 36:39-44.