On the Nature of NAA Diffusion and the Apparent Viscosity Inside Neurons of the Central Nervous System

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Abstract Herein we report the first determination of the apparent viscosity of neuronal cytoplasm in the mammalian brain *in vivo* and *in situ*. This represents an important step toward developing a quantitative understanding of the biophysical determinants of the MR diffusion signal. The diffusion-sensitized NAA MR signal depends upon *b* in a non-monoexponential manner. We provide a quantitative description of this dependence using a theoretical model in which NAA is confined to multiply-oriented neuronal fibers. We find that diffusion parallel to fibers, $ADC_{\parallel}$, is 0.36±0.06 $\mu m^2/ms$, and diffusion perpendicular to fibers, $ADC_{\perp}$, is severely reduced at $(2.3\pm7.6)\times10^{-4}$ $\mu m^2/ms$. From $ADC_{\parallel}$, the apparent viscosity of the neuron cytoplasm is estimated to be two-fold larger than dilute aqueous solution.

Introduction In the brain, NAA exists almost exclusively within neurons (1). Diffusion of this molecule occurs within axons and dendrites, and in large voxels containing gray matter or multiply-oriented fibers of white matter, may be modeled as diffusion within randomly oriented cylinders. This problem is mathematically identical to the problem of ^{3}He gas diffusion in lung airways (2). Herein we apply the method developed in (2) to extract information on anisotropic NAA diffusivities (parallel to neuronal axis, $ADC_{\parallel}$, and perpendicular to axis, $ADC_{\perp}$) from measurements in macroscopically isotropic structures.

Experimental Diffusion-weighted LASER spectroscopy of 5 rats was performed at 4.7 T using a 1.5 cm diameter surface coil. $ADC_{\parallel}$ and $ADC_{\perp}$ values were determined according to reference (2) to the NAA methyl ^{1}H MR signal decay versus *b*. Diffusion-weighted PRESS spectroscopy, localized to the corpus callosum, was also performed at 1.5 T on 2 human subjects. In the humans, $ADC_{\parallel}$ and $ADC_{\perp}$ values were obtained by aligning the diffusion-sensitizing gradients parallel and perpendicular, respectively, to the known direction of the neuronal fibers.

Table 1. Mean ADC ± uncertainties ($\mu m^2/ms$)

Subjects	$ADC_{\parallel}$	$ADC_{\perp}$
5 Rats	0.36 ± 0.06	$(2.3 \pm 7.6) \times 10^{-4}$
2 Humans	0.32 ± 0.07	0.13 ± 0.06

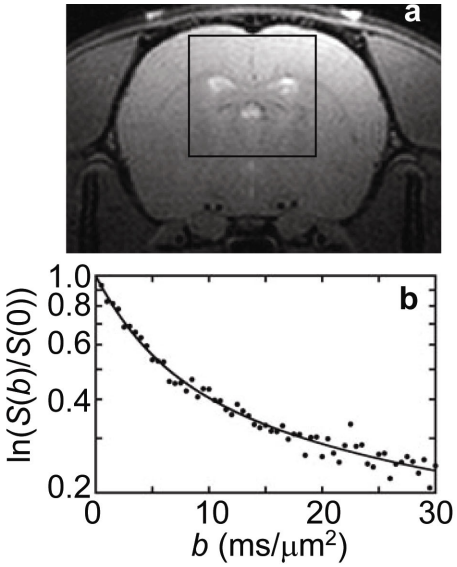

Figure 1. (**a**) Central plane of one voxel. (**b**) NAA methyl ^{1}H MR signal versus *b*. Solid line is a fit of the reference (2) formula.

Results Figure 1a shows a representative rat volume element selected for localized spectroscopy. An example fit to the data is plotted in Figure 1b. Table 1 summarizes the $ADC_{\parallel}$ and $ADC_{\perp}$ values obtained from five rats and two human volunteers.

Discussion To the extent that diffusion parallel to the neuronal fiber axes is expected to be largely unrestricted by membrane barriers, $ADC_{\parallel}$ reflects the apparent viscosity of the intracellular space. Therefore the $ADC_{\parallel}$ we obtain *in situ* is a close approximation of D^{intra}, the true diffusion coefficient in cytoplasm. (Table 2 demonstrates that the D^{intra}/D^{free} ratios measured using various model systems reflect similar increases in apparent viscosity relative to free media.) This measurement provides an important parameter in the development of a quantitative model of the biophysics underlying the MR diffusion signal in the mammalian central nervous system.

Acknowledgement Supported by NIH grants F32 43010 and R01 35912 and R24 CA83060. **References 1.** Urenjak et al. *J Neurosci* 13:981-989, 1993 **2.** Yablonskiy et al. PNAS 99:3111-3116, 2002 **3.** Verkman, *TIBS* 27:27-33, 2002 **4.** Popov and Poo, *J Neurosci* 12:77-85, 1992 **5.** Albritton et al. *Science* 258:1812-1815, 1992 **6.** Sehy et al. *MRM* 48:42-51, 2002 **7.** Mastro et al. *PNAS* 81:3414-3418, 1984 **8.** Caille and Hinke, *Can J Physiol Pharmacol* 52:814-828, 1974 **9.** Koike and Nagata, *J Physiol* 295:397-417 1979

Table 2. D^{intra}/D^{free} ratios

Compound	MW (Da)	D^{intra}/D^{free}	Experimental Source
BCFEF	520	0.27	Flourescence, cultured cells (3)
2 nM Dextran	~5000	0.39	Flourescence, *Xenopus* neuron (4)
IP_3	174	0.39	Radiotracer, oocyte extract (5)
Various	18-4600	0.45	MR, *Xenopus* oocyte (6)
NAA	**175**	**0.46**	**Current study**
PCAOL	170	0.52	ESR, mammalian cells (7)
Various	18-182	0.53-0.58	Radiotracer, muscle fiber (8)
Acetylcholine	250	0.58	Radiotracer, *Aplysia* axon (9)