NeuroImage xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

NeuroImage

journal homepage: www.elsevier.com/locate/ynimg

Temperature dependence of water diffusion pools in brain white matter

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ARTICLE INFO

Article history:
Received 17 April 2015
Accepted 25 November 2015
Available online xxxx

Keywords:
White matter
Diffusion
Activation energy
Myelin
Microtubules

ABSTRACT

Water diffusion in brain tissue can now be easily investigated using magnetic resonance (MR) techniques, providing unique insights into cellular level microstructure such as axonal orientation. The diffusive motion in white matter is known to be non-Gaussian, with increasing evidence for more than one water-containing tissue compartment. In this study, freshly excised porcine brain white matter was measured using a 125-MHz MR spectrometer (3 T) equipped with gradient coils providing magnetic field gradients of up to 35,000 mT/m. The sample temperature was varied between −14 and +19 °C. The hypothesis tested was that white matter contains two slowly exchanging pools of water molecules with different diffusion properties. A Stejskal–Tanner diffusion sequence with very short gradient pulses and *b*-factors up to 18.8 ms/μm^2 was used. The dependence on *b*-factor of the attenuation due to diffusion was robustly fitted by a biexponential function, with comparable volume fractions for each component. The diffusion coefficient of each component follows Arrhenius behavior, with significantly different activation energies. The measured volume fractions are consistent with the existence of three water-containing compartments, the first comprising relatively free cytoplasmic and extracellular water molecules, the second of water molecules in glial processes, and the third comprising water molecules closely associated with membranes, as for example, in the myelin sheaths and elsewhere. The activation energy of the slow diffusion pool suggests proton hopping at the surface of membranes by a Grotthuss mechanism, mediated by hydrating water molecules.

Introduction

In recent years, diffusion-weighted magnetic resonance (DW-MR) measurements in brain white matter (WM) have gained increasing interest among neuroscientists and clinicians. Investigation of water diffusion in WM using MR provides unique insights into cellular-level microstructure, such as axonal orientation, and can provide information regarding the well-being and pathology of the tissue (Horsfield and Jones, 2002; Werring et al., 1999). Diffusion measurements have also been extensively used in the context of diffusion tensor imaging (DTI) (Basser et al., 1994) and other more complex and powerful methods to reveal fiber orientation in WM (Tuch et al., 2002; Wedeen et al., 2005).

DW-MR has shown great promise in characterizing WM; nevertheless, understanding of the biophysical characteristics of water diffusion in WM has been elusive. Diffusional decay in WM has been shown to be non-monoexponential (Assaf and Cohen, 2000; Beaulieu and Allen, 1994; Clark and Le Bihan, 2000; Henkelman et al., 1994; Niendorf et al., 1996), and various models have been put forth to explain the relationship between water mobility and different features of the DW signal. Most models involve cylindrical restriction for intra-axonal water in the direction perpendicular to the main fiber orientation and hindrances for extra-axonal water (Alexander, 2008; Assaf et al., 2004, 2008). Stanisz et al. (Stanisz et al., 1997) showed that a model consisting of ellipsoids and spheres with partially permeable membranes can describe the data. It has also been a common practice to treat diffusion as arising from two pools of water exchanging slowly compared to the diffusion time employed (Clark and Le Bihan, 2000). In a recent paper, Le Bihan (Le Bihan, 2007) assigned the more slowly diffusing pool to the hydration-layer water close to the membranes. The challenge of effective and realistic modeling is underlined by a recent article (Panagiotaki et al., 2012) comparing 47 different models found in the literature.

Lately, many studies of non-Gaussian diffusion in WM have focused mainly on diffusion properties perpendicular to the fiber directions (Barazany et al., 2009; Bar-Shir et al., 2008; Bar-Shir and Cohen, 2008; Cohen and Assaf, 2002; Ong et al., 2008; Ong and Wehrli, 2010). While methods involving measurements perpendicular to the fiber direction have claimed success in estimating axonal diameters, so far these studies have implicitly treated layers of myelin as ultimate restrictive barriers and did not consider a possible multi-compartmental nature of diffusion data that may be intrinsic to the WM.

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http://dx.doi.org/10.1016/j.neuroimage.2015.11.064
1053-8119/

An unexplored area related to WM diffusivity is its temperature dependence. For large temperature ranges, the temperature dependence of water diffusivity (in pure water) has been reported to fit a Speedy–Angell power-law (Holz et al., 2000). However, at temperatures near 0 °C, the temperature dependence of water can be approximated by an Arrhenius equation,

$$D(T) = D_0 \exp(-E_a/RT) \quad (1)$$

where E_a is the activation energy of the apparent diffusion coefficient $D(T)$, D_0 is the prefactor, R is the universal gas constant, and T the temperature in Kelvin. For bulk water, E_a has been reported to lie between 18.8 and 19.2 kJ/mol at room temperature (Gillen et al., 1972; Wang et al., 1953). In tissues containing multiple water pools, measuring the diffusion decay against temperature can shed light on the respective environment of the water pools and their interaction. The complementary information could come from three different perspectives. First, according to Kärger's equation (Kärger et al., 1988), if exchange plays a significant role, the apparent volume fractions of the two pools obtained from a biexponential fit should also change with temperature. Second, the activation energies of different water pools should also provide more insight into the environment in which they reside. Third, in brain WM layers of compact myelin wrapped by oligodendrocytes around the axon form a unique cellular structure lacking cytoplasm, in which a small fraction of the water described as 'myelin water' is found in thin layers (≈2–4 nm thick (De Felici et al., 2008)) between apposed membranes held tightly together by a regular pattern of myelin basic proteins (MBP) and proteolipid proteins (PLP) (see *compact myelin* in Fig. 6). The confinement of myelin water in such narrow environments is expected to result in a depression of the freezing point (Ashworth and Abeles, 1984; Morishige and Kawano, 1999; Petrov and Furó, 2009).

To better understand the multi-exponential decay of diffusion data, this study measured diffusion with very high *b*-factors yet at short diffusion time (Δ) and echo time (TE). A short Δ avoids confounding of the signal by exchange between different pools, and a short TE reduces inaccurate estimation of the volume fraction due to potential differences in spin–spin relaxation times (T_2) of different diffusing pools. To circumvent restriction effects associated with layers of myelin, the measurements were performed parallel to the fiber orientation. They were also repeated at different temperatures, allowing the estimation of the activation energies of water diffusion for different water pools.

Methods

Sample preparation

In preliminary measurements performed on formalin-fixed postmortem human corpus callosum (Dhital et al., 2011), we observed a lower freezing transition (−18 to −20 °C) than that reported by Escanyé et al. for biological tissues (−4 to −9 °C) (Escanyé et al., 1984), suggesting an effect of fixation on the macroscopic water environment (Morishige and Kawano, 1999). To avoid such effects, fresh unfixed tissue was used in this study. Fresh tissue undergoes autolytic degradation from the onset of death. Although, freezing the sample would stop such degradations, MBP rapidly degrades after thawing (Ansari et al., 1975), whereas it is relatively resistant to autolytic changes in unfrozen myelin, for which decompaction occurs along the interperiod lines (extracellular) (Ansari et al., 1976). Autolytic changes in WM lead to decompaction of myelin as reported from electron microscopy studies: During 6 h at +4 °C mitochondria are swollen, whereas at +25 °C focal splittings of myelin are observed, which form a network-like splitting after 24 h at +25 °C (Hukkanen and Röyttä, 1987). In addition, ATP depletion leads to swelling of the glial cells within an hour after slaughtering (Jurkowitz-Alexander et al., 1992).

On different days, four freshly cut pig brains were acquired from a local slaughterhouse. For each measurement, within minutes of sacrifice the brain sample was immersed in phosphate buffered solution (PBS, pH 7.4). During transportation, the temperature was maintained between 0 and +4 °C. At the laboratory, a small cylindrical piece of corpus callosum was dissected (4 mm diameter, 4 mm height), excess PBS was lightly soaked up with cotton and the sample was placed in a nuclear magnetic resonance (NMR) tube. To maintain humidity, the tube was closed at the top with a small piece of cotton soaked in PBS and sealed with wax. The sample was placed in the NMR tube such that the callosal fibers were aligned with the main magnetic field and the pulsed field gradient direction of the spectrometer. All measurements were completed within 9 h of the slaughter of the animal, including ca. 1.5 h for transportation and 1.5 h for sample preparation, tuning and matching. The total time duration for the experiment was ca. 4 h. The sample remained above +4 °C for about 2.5 h. Under these conditions, it is assumed that the myelin sheath was only affected by focal decompaction.

Data acquisition

All NMR data were acquired using a homebuilt FEGRIS-NT 125-MHz spectrometer equipped with a unidirectional ultra-high-intensity magnetic field gradient system that has a peak gradient strength of 35,000 mT/m parallel to the main magnetic field (Galvosas et al., 2001; Kärger et al., 1995). The temperature of the sample in the bore was controlled using a stream of gaseous nitrogen evaporated from a liquid reservoir and a built-in heater/thermometer regulation system. The initial system temperature was set to +19 °C. To minimize the experimental time in this study involving fresh tissue samples, all the pre-measurement steps such as tuning, matching, resonance frequency setting, and determining the optimal pulse durations were performed at this initial temperature.

We note that the electrical properties of water or saline solutions change upon freezing. However, we did not observe a significant effect on the tuning of the resonance circuit of the detector coil at the freezing point, presumably because of the small size of the RF coil. The wavelength in brain tissue is approx. 30 cm at 125 MHz (Driesel et al., 2005), which exceeds the diameter of the RF coil (approx. 7.5 mm) by a factor of 40. Under these conditions, the conductivity of the sample is insufficient to 'bleed off' appreciable power from the current in the coil, and sample losses are, thus, negligible (Chen and Hoult, 1989). Above the freezing transition, there was a negligible frequency shift of less than 40 Hz when lowering the temperature by 25 °C. Around the freezing transition, a larger shift is expected due to the more pronounced change in dielectric properties around the phase transition. Experimentally, a shift of less than 50 Hz was observed upon a temperature reduction by 5 °C in this regime. In a preliminary investigation with formalin-fixed samples, the effects of detuning were not found to change our observations (Dhital et al., 2011).

All samples demonstrated a small increase in the signal intensity with decreasing temperature in agreement with Curie's law (Fig. 1), which was also observed in a preliminary experiment with formalin-fixed samples (Dhital et al., 2011) for a temperature ranging from +20 °C to the freezing transition (−18 °C).

All RF pulses were rectangular in shape. For different samples the excitation pulse duration was between 3.75 and 4 μs, and the refocusing pulse had a duration of 7.5 to 8 μs. Free induction decays (FID) were measured with an initial dead time of 125 μs, with intervals of 5 μs between successive data points. The temperature was lowered by 1 °C at each step, and the FID was recorded in intervals of 30 s. Since the solid-state NMR signal of ice is not detected in the FID signal at 125 μs, an average of the first four data points from the FID signal (between 125 μs and 140 μs) was taken as a proxy for 'liquid' water. The fraction of 'unfrozen' water at the freezing transition was calculated as the ratio between the signal intensities immediately before and after the freezing transition (see Fig. 1). Each time the temperature was lowered, the FID measurements were repeated until the mean of these four points between two successive measurements was within 1% of each

Please cite this article as: Dhital, B., et al., Temperature dependence of water diffusion pools in brain white matter, NeuroImage (2015), http://dx.doi.org/10.1016/j.neuroimage.2015.11.064