NeuroImage 129 (2016) 414–427

Contents lists available at ScienceDirect

NeuroImage

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

Full Length Articles

In vivo observation and biophysical interpretation of time-dependent diffusion in human white matter

Els Fieremans [a,*], Lauren M. Burcaw [a], Hong-Hsi Lee [a], Gregory Lemberskiy [a], Jelle Veraart [a,b], Dmitry S. Novikov [a]

[a] Bernard and Irene Schwartz Center for Biomedical Imaging, Department of Radiology, New York University School of Medicine, New York, NY 10016, USA
[b] iMinds Vision Lab, Department of Physics, University of Antwerp, Antwerp, Belgium

ARTICLE INFO

Article history:
Received 28 July 2015
Accepted 8 January 2016
Available online 20 January 2016

Keywords:
Diffusion MRI
Diffusion tensor imaging
STEAM sequence
Time-dependent diffusion
Hindered diffusion
White matter

ABSTRACT

The presence of micrometer-level restrictions leads to a decrease of diffusion coefficient with diffusion time. Here we investigate this effect in human white matter *in vivo*. We focus on a broad range of diffusion times, up to 600 ms, covering diffusion length scales up to about 30 μm. We perform stimulated echo diffusion tensor imaging on 5 healthy volunteers and observe a relatively weak time-dependence in diffusion transverse to major fiber tracts. Remarkably, we also find notable time-dependence in the longitudinal direction. Comparing models of diffusion in ordered, confined and disordered media, we argue that the time-dependence in both directions can arise due to *structural disorder*, such as axonal beads in the longitudinal direction, and the random packing geometry of fibers within a bundle in the transverse direction. These time-dependent effects extend beyond a simple picture of Gaussian compartments, and may lead to novel markers that are specific to neuronal fiber geometry at the micrometer scale.

Introduction

The unique advantage of diffusion-weighted magnetic resonance imaging (dMRI) arises from the sensitivity of water diffusion to the micrometer-level structure of its surrounding environment. In biological tissues, restrictions such as cell walls provide the basis for contrast in dMRI, and particularly, in diffusion tensor imaging (DTI) (Basser, 1995; Beaulieu, 2002). This contrast holds the promise of probing neuronal tissue structure at the scales of about three orders of magnitude below the nominal clinical MRI resolution. From the physics standpoint, this involves quantifying the relevant *length scales*, such as the compartment (cell) size, or the cell packing correlation length.

There are two physically distinct ways of being sensitive to the cellular length scale: by varying the diffusion wave vector q, or by varying the diffusion time t, as illustrated in Fig. 1 of (Burcaw et al., 2015). The q-method, based on Callaghan's "diffusion diffraction" effect (Callaghan et al., 1991), measures the diffusion signal (the propagator) in the narrow pulse limit as function of q, and the length scale (the fully restricted pore size) is given by the inverse of the characteristic q value for which the propagator experiences oscillations. Unfortunately, given ~1 μm-diameter axons and dendrites, the required q values are prohibitively large for *in vivo* human measurements.

Instead, here our aim is to derive the relevant length scale(s) in human white matter (WM) by varying t, and studying the time-dependence $D(t)$ of the diffusion coefficient (more generally, of the diffusion tensor eigenvalues). This formally amounts to a $q \to 0$ measurement as the diffusion coefficient is proportional to a derivative of the dMRI signal at $q = 0$, which thereby makes our approach clinically feasible. Since the diffusion coefficient in a given direction x is a measure of the mean squared displacement, i.e. $D(t) = \langle (x(t) - x(0))^2 \rangle / 2t$, the length scale probed by water molecules may be adjusted by varying t. With increasing t, water molecules encounter more hindrances and restrictions to their diffusion paths, such as cellular walls and myelin, and therefore the resultant measured diffusion coefficient will decrease (Mitra et al., 1992; Novikov et al., 2014).

While time-dependence of the diffusion coefficient in mammalian WM has been clearly demonstrated at short times (~ 1 ms) (discussed in more detail below), *in vivo* evidence for the time-dependence using pulse gradient spin echo (PGSE) methods over clinically feasible diffusion time ranges ($t > 20$ ms) has been inconsistent. *In vivo* studies of brain, such as healthy and ischemic feline brain tissue (van Gelderen et al., 1994) yielded no change in the mean diffusivity with respect to t for a wide range encompassing 20–2000 ms. Nor was time-dependence observed *in vivo* in the mean diffusivity of human genu at relatively short times ($t = 8$ – 80 ms) (Clark et al., 2001) or in the longitudinal or transverse diffusivity within the human corticospinal tract for even longer times ($t = 64$ – 256 ms) (Nilsson et al., 2009). On the other hand, time-dependent diffusion has been observed *in vivo* in the corpus callosum, corona radiata, and brainstem of human subjects at times ranging from 40 to 800 ms (Horsfield et al., 1994). Furthermore, *ex vivo* studies in frog sciatic nerve with diffusion times of 2 ms and 28 ms (Beaulieu and Allen, 1996), bovine optic nerve with diffusion times ranging from 8 to 30 ms (Stanisz et al., 1997), optic and sciatic

* Corresponding author.
E-mail address: els.fieremans@nyumc.org (E. Fieremans).

http://dx.doi.org/10.1016/j.neuroimage.2016.01.018
1053-8119/

nerves with diffusion times from 3.7 ms to 99.3 ms (Bar-Shir and Cohen, 2008), as well as bovine optic nerve and rat spinal cord and brain with diffusion times from 40 to 250 ms (Assaf and Cohen, 2000) have shown a clear dependence of $D(t)$ on time in longitudinal and/or transverse direction. More recently, Kunz et al. (2013) imaged the rat corpus callosum *in vivo* at t ranging from 9 to 24 ms and found time-dependent diffusion in both longitudinal and transverse directions.

Oscillating gradient spin echo (OGSE) diffusion-weighted sequences are able to probe shorter diffusion times compared to conventional PGSE, and have demonstrated time-dependent diffusion in the brain. An *in vivo* oscillating gradient study of the rat cortex (Does et al., 2003) at frequencies up to 500 Hz, which correspond to very short $t \gtrsim 1$ ms, shows a clear time-dependence in the mean diffusivity in both normal live and post-mortem globally ischemic rat cortex. Later work using OGSE with corresponding effective diffusion times (1 – 5 ms) also demonstrated time-dependence in *ex vivo* rat WM tracts (Xu et al., 2014). In humans, Baron et al. (Baron and Beaulieu, 2014) combined OGSE (25 and 50 Hz) and PGSE methods (t = 20 and 40 ms) for a total diffusion time range of 4 to 40 ms, and found eight major WM tracts and two deep gray matter areas to exhibit time-dependent diffusion. Van et al. (2014) have seen a similar effect with OGSE in human corpus callosum in the frequency range 18–63 Hz. Furthermore, recent work using double PFG MR indirectly points at the possibly non-Gaussian (time-dependent) nature of diffusion in the extracellular space of WM with increasing diffusion times from 25 to 100 ms (Shemesh and Cohen, 2011).

Here we report the observation of time-dependent diffusion *in vivo* for relatively long diffusion times, t = 45 – 600 ms, on a standard clinical scanner using STimulated Echo Acquisition Mode (STEAM)-DTI, and discuss the biophysical origin of this phenomenon. STEAM-DTI measurements were performed on 5 healthy volunteers and were calibrated on a gel phantom over the whole time range. Pronounced time-dependence in the longitudinal diffusivity and less pronounced time-dependence in the transverse diffusivity were found in both anatomically based WM regions and in fractional anisotropy (FA) thresholded regions.

The biophysical origin of the observed time-dependence, as discussed below, reflects the non-Gaussian nature of diffusion in at least one tissue compartment (in either direction). In all cases, both longitudinal and transverse diffusivities approach a finite tortuosity limit (*i.e.* diffusion is not anomalous (Bouchaud and Georges, 1990)), with a slow transient part that is best described by a power-law behavior (Novikov et al., 2014; Burcaw et al., 2015). We argue that the origin of this behavior is likely due to randomly placed (short-range disordered) hindrances and restrictions to diffusion in both parallel and transverse directions. Interestingly, the biological sources of this short-range disorder may be qualitatively distinct: structural disorder along the axons such as, e.g., varicosities for diffusion in the longitudinal direction, and the random packing geometry of fibers within a bundle for diffusion in the transverse direction. This picture is corroborated by the estimated correlation length scales in the range of a few microns in both directions.

Methods

In vivo measurements

Diffusion measurements were performed on 5 healthy volunteers (4 males and 1 female) ranging in age from 25 to 41 years old, on a 3 T Siemens Tim Trio (Erlangen, Germany) equipped with a 32-channel head coil and a maximum gradient strength of 40 mT/m during two 1-h scans utilizing the STEAM-DTI sequence as provided by the vendor (WIP 511E). One volunteer was unable to be present for scan 2. Each diffusion sequence acquired $b = 0$ (5 averages) and $b = 500$ s/mm^2 images along 20 diffusion directions, with an isotropic voxel size of (2.7 mm)^3, and a field of view (FOV) of (221 mm)^2. A slab of 15 axial slices was aligned parallel to the anterior commissure (AC) – posterior commissure (PC) line and centered such that the entire corpus callosum would be imaged. Both scans were focused on varying the diffusion time, $t = \Delta$ (interpulse duration) ranging from 45 to 400 ms (scan 1) and from 55 to 600 ms (scan 2), while keeping δ, the pulse duration of the diffusion gradients, and TE, the echo time, constant. The parameters in these two scans were the following: δ = 20 ms, TE = 100 ms, TR = 7000 ms for t between 45 and 400 ms, with TR increasing to 10,200 ms at t = 600 ms. A summary of the specific parameters for each scan session can be found in Table 1.

Our *in vivo* measurement was calibrated by performing the same measurements on a nickel-doped agarose gel phantom, made by dissolving 1.4% agarose and 9 g/L of sodium chloride in distilled water and adding 2 mM Nickel and 6 g/L sodium azide, as described in (Lavdas et al., 2013). The gel was kept in a small cylindrical jar of roughly 10 cm long with a diameter of 6.5 cm.

Parameter map construction

In order to reduce the effect of Gibbs ringing surrounding the ventricles (Veraart et al., 2015), Gaussian filtering was applied to the dMRI images with a full width half maximum of 1.25 voxels and a window size of 5×5 voxels. To avoid cerebrospinal fluid (CSF) signal contamination in WM neighboring the ventricles during smoothing, a CSF mask was constructed via FSL's automated segmentation tool, FAST (Zhang et al., 2001) and used to separate the CSF from the rest of the brain parenchyma. These two resultant images (CSF and brain parenchyma) were smoothed separately using the parameters described above, which amounted to reducing the smoothing window size when getting close to the border, and recombined post smoothing.

Eigenvalues were calculated from the diffusion tensors that were estimated via a weighted linear least squares (WLLS) routine. The weights were derived from the DTI estimation by the unweighted LLS estimator (Veraart et al., 2013) and a corrected full b-matrix which incorporated the effective gradient from the diffusion, imaging gradients, and radio-frequency (RF) pulse magnetization inversions (Lundell et al., 2014; Sigmund et al., 2013). For the $b = 0$ image, the actual b-value (obtained from the trace of the b-matrix) varied from 2 s/mm^2 for Δ = 45 ms, up to 67 s/mm^2 for Δ = 500 ms, while the diffusion gradients were adjusted for the $b = 500$ image such that the actual $b = 500$ s/mm^2 (Lundell et al., 2014).

Previous work (Veraart et al., 2013) has determined that the WLLS estimator is unbiased if the Rician distributed data has SNR > 2. SNR was estimated in the $b = 0$ image via the ratio of a region of interest (ROI) placed in the splenium to an ROI placed in the background noise and corrected for the Rician statistics. We found that SNR varied from 15 for our shortest time down to 6 for our longest diffusion time at $b = 0$. Since the observed longitudinal and transverse diffusivities did not exceed 2 μm^2/s, the SNR on the DW images is sufficiently high to avoid a Rician noise bias.

The output parameter maps include the eigenvalues of the diffusion tensor **D**, the $S_{b=0}$ image, and fractional anisotropy (FA). Given the strong anisotropy of diffusion in major fiber tracts, it is natural to define the longitudinal diffusivity

$$D_{||}(t) = \lambda_1 \tag{1}$$

in terms of the principal eigenvalue λ_1 of the diffusion tensor (the DTI eigenvalues are sorted according to $\lambda_3 \le \lambda_2 \le \lambda_1$), and the transverse diffusivity

$$D_{\perp}(t) = (\lambda_2 + \lambda_3)/2 \tag{2}$$

as the axially symmetric component of the diffusion tensor projected onto the plane transverse to its principal eigenvector (tract direction).