

Changes to the fractional anisotropy and mean diffusivity of in vivo rat brain measured at short effective diffusion-times

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Introduction: It has long been hypothesised that the restricted (or hindered) motion of water molecules is responsible for the anisotropic image contrast in diffusion-tensor MRI. It has also been recognised that by varying the length or separation of the motion-probing gradients (MPGs) in a pulsed-gradient spin-echo (PGSE) sequence, it should be possible to alter the contrast of *in vivo* images and thus common measures of diffusion anisotropy to probe tissue microstructure. Unfortunately, previous efforts to demonstrate this were unable to find *in vivo* evidence for the restricted-diffusion hypothesis (eg [1]).

Recent work, using a technique that employs sinusoidally oscillating MPGs added to a standard spin-echo sequence, has demonstrated some of the hallmarks of restricted diffusion for *in vitro* samples, and normal and diseased rat brain [2-4]. In the only previous application of the technique to diffusion tensor imaging (DTI), it was found that the fractional anisotropy (FA) of white matter in an *ex vivo* monkey brain was clearly dependent on MPG frequency [5]. The experimental results were in agreement with computer simulations from a restricted diffusion model and therefore the technique is expected to be a useful method for probing microstructure. However, the technique has never been applied to examine how the diffusion tensor is altered for *in vivo* DTI. In this study, an oscillating MPG sequence was applied to investigate changes to the apparent diffusion tensor, FA and mean diffusivity (MD) measured for *in vivo* rat brain as the MPG frequency is increased (or equivalently, the diffusion-time is decreased).

Methods: Male Sprague-Dawley rats (200-300 gm) were anaesthetised with isoflurane and fixed in a cradle with bite and ear bars. The cradle was inserted into the bore of a 7T magnet and then rotated so that the animal was upside-down to minimise the effects of respiratory motion. Rectal temperature was maintained at around 37°C with heated air throughout the experiment. All MRI data were acquired on a 7 T MRI system (Magnet: Kobelco, Japan; Console: Bruker Avance I, Germany) equipped with an actively-screened gradient system (Bruker BGA12). A volume coil (diameter 72 mm, Bruker) was used for transmission and a 2-channel phased-array surface coil (Rapid Biomedical, Germany) was used for signal reception.

A sagittal slice through the rat cerebellum and corpus callosum was selected for imaging. These two areas contain most of the white matter in the rat brain and therefore show the highest degree of diffusion anisotropy. Data was obtained with a four-shot SE-EPI sequence that had cosinusoidal MPG waveforms placed on either side of the refocussing pulse [2-5]. Images were acquired for 30 evenly distributed MPG directions with b-values of 0 & 1000 s/mm² and MPG frequencies of 33.3 & 66.6 Hz. Other parameters were TR = 3 s, TE = 79 ms, MPG duration = 30 ms, spatial resolution = 0.2 mm x 0.2 mm x 1 mm, slice thickness = 1 mm and scan time = 18 min. T2-weighted anatomical images (TR = 3 s, TE = 60 ms) of the same slice were also acquired with a standard SE sequence.

The data was analysed offline using purpose-written Matlab code. No spatial smoothing was applied before the diffusion tensor was estimated pixel-by-pixel from the raw images. Regions-of-interest (ROIs) in the corpus callosum and cerebellar white matter were drawn by hand on the anatomical images (Fig. 1). ROIs in the visual cortex and cerebellar grey matter were also drawn for comparison. The FA and MD of all pixels in each ROI were grouped according to MPG frequency and paired t-tests were then performed to investigate the dependence on frequency.

Results: Analysis of the DTI data found higher FAs in the corpus callosum and cerebellar white matter ROIs than in the visual cortex and cerebellar grey matter ROIs (Fig. 2a). Paired-t tests for each ROI only found a significant decrease of the FA for the corpus callosum and cerebellar white matter after the effective diffusion-time was decreased from 7.5 ms to 3.75 ms (or, the MPG frequency was increased from 33.3 Hz to 66.6 Hz). Tests of the MD in each ROI found significant increases with decreasing diffusion-time for the cerebellar white and grey matters as well as for the visual cortex (Fig. 2b). These results are consistent with the restricted/hindered

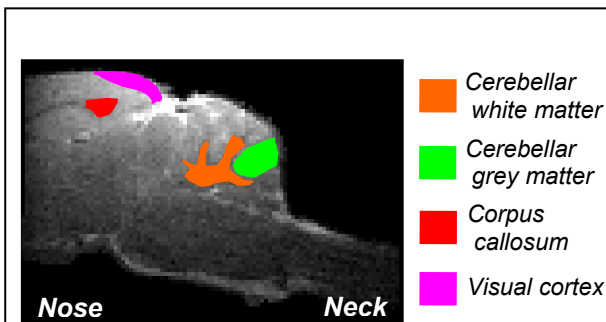


Figure 1 Sagittal slice through the rat brain with the regions-of-interest superimposed.

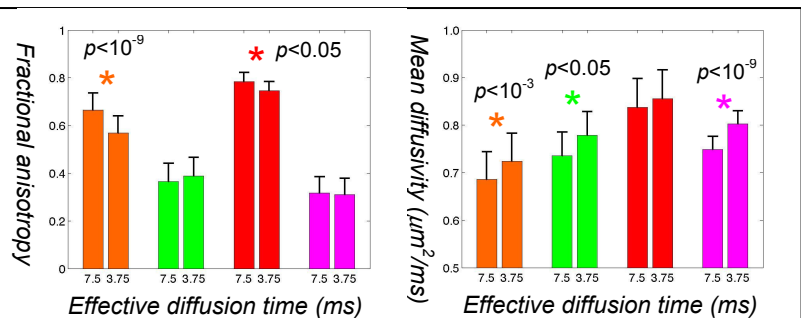


Figure 2 (left) Significant decreases of the FA were observed in cerebellar white matter and the corpus callosum. (right) Significant increases of the MD occurred in cerebellar white and gray matters as well as the visual cortex.

diffusion model.

Discussion: Further experiments at higher gradient strengths will enable *in vivo* investigation of FA and MD at much lower diffusion times and therefore allow smaller tissue structures to be probed. Example applications include examination of fibre crossings and the measurement of changes to axonal diameter. Furthermore, it is anticipated that differences in normal and pathological *in vivo* tissue structure (eg neurodegenerative diseases) can be compared with this technique.

References: [1] Clark *et al*, *Magn Reson Med*, 45, 1126-9 (2001); [2] Does *et al*, *Magn Reson Med*, 49, 206-215 (2003); [3] Parsons *et al*, *Magn Reson Imaging*, 21, 279-285 (2003); [4] Colvin *et al*, *Cancer Res*, 68, 5941-7 (2008); [5] Xu *et al*, *Proc ISMRM*, 4038 (2010).