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Systematic changes to the apparent diffusion tensor of in vivo rat brain measured with an oscillating-gradient spin-echo sequence

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ABSTRACT

As the oscillating gradient spin-echo sequence has shown promise as a means to probe tissue microstructure, it was applied here to diffusion-tensor imaging of in vivo rat brain. The apparent diffusion tensor (ADT) was estimated for motion-probing gradient (MPG) frequencies in the range 33.3–133.3 Hz, and regions-of-interest (ROIs) in the corpus callosum (CC), visual cortex (VC), cerebellar white matter (CBWM) and cerebellar grey matter (CBGM) were selected for detailed analysis. There were substantial, approximately linear changes to the ADT with increasing MPG frequency for all four ROIs. All ROIs showed clear increases in mean diffusivity. CBWM had a substantial decrease in fractional anisotropy, whereas the CC and VC had minor increases of the same parameter. All eigenvalues of the ADT tended to increase with frequency for the CBWM, CBGM and VC, but only the principal eigenvalue increased strongly for the CC. On the other hand, there was no evidence that the orientation of the principal eigenvector varied systematically with MPG frequency for any of the ROIs. The relationship between the behaviour of the eigenvalues and the behaviours of the mean diffusivity and fractional anisotropy is investigated in detail. Pixelwise linear fits to the MD from individual animals found elevated changes across the cerebellum. The data acquired for this work encompassed a range of effective diffusion-times from 7.5 ms down to 1.875 ms, and some ideas on how the results might be used to extract quantitative information about brain tissue microstructure are discussed.

Introduction

Many studies with diffusion-weighted MRI have indicated that manipulating the parameters of the motion-probing gradients (MPGs) offers a noninvasive means to probe in vivo tissue microstructure. One parameter routinely modified to alter image contrast is the orientation of the MPGs. Cycling the MPG direction around a hemisphere while preserving other parameters is used in diffusion-tensor MRI (DTI) (Basser et al., 1994) and other techniques like q-ball (Tuch, 2004) and HARDI (Tuch et al., 2002) to investigate tissue anisotropy. Another parameter with the potential to modify image contrast is the diffusion-time. Modulating the diffusion-time is often cited as a means to investigate restricted or hindered diffusion in complex media, but it is widely neglected in clinical situations because evidence for distinct, unequivocal contrast changes in vivo is sparse.

Most studies investigating the effects of varying diffusion-time on image contrast in diffusion-weighted MRI have been performed with conventional pulsed-gradient spin-echo (PGSE) and pulsed-gradient stimulated-echo (PGStE) sequences. Using these sequences, changes to the signal with decreasing diffusion-time have been found in a variety of biological systems, including in vitro cell cultures (Pilatus et al., 1997), ex vivo rat brain (Assaf and Cohen, 1998) and in vivo radiation-induced tumors (Helmer et al., 1995). In vivo experiments on normal tissue have also been performed, but, with two exceptions, the studies were limited to diffusion-times of 8 ms or longer and no significant signal changes were observed (Clark et al., 2001; Le Bihan et al., 1993; Moonen et al., 1991; Niendorf et al., 1996; van Gelderen et al., 1994). One of the exceptions reported changes for diffusion-times in the range 33.3–793.3 ms (Horsfield et al., 1996). However, as the *b*-value of a sequence is not independent of the diffusion-time and no precautions seem to have been taken to keep the *b*-value constant, other researchers have questioned whether

Abbreviations: OGSE, oscillating gradient spin-echo; DTI, diffusion-tensor imaging; PGSE, pulsed-gradient spin-echo; PGStE, pulsed-gradient stimulated-echo; MPG, motion-probing gradient; ADT, apparent diffusion tensor; ADC, apparent diffusion coefficient; MD, mean diffusivity; FA, fractional anisotropy; EV, eigenvalue; ROI, region of interest; CBWM, cerebellar white matter; CBGM, cerebellar grey matter; CC, corpus callosum; VC, visual cortex; CBGr, cerebellar granular layer.

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the observed signal changes can be unambiguously attributed to a diffusion-time dependent effect (Clark et al., 2001; Does et al., 2003). In the second exception, a bipolar MPG was placed in only the first half of the echo-time so that diffusion-times as low as 1.6 ms were possible (Niendorf et al., 1994). At a constant *b*-value of 210 s/mm^2, the signal was found to decrease as the diffusion-time was reduced from 5.9 to 3.4 ms, suggesting that diffusion-time related contrast changes can be observed in vivo if the diffusion-time is less than around 5 ms, something that is difficult to achieve with standard PGSE and PGStE sequences.

As an alternative to PGSE and PGStE, the oscillating-gradient spin-echo (OGSE) sequence has been proposed as a means to reach diffusion-times of less than 5 ms (Gross and Kosfeld, 1969; Schachter et al., 2000). While OGSE was originally implemented with sinusoidal MPGs, more recent versions employ an apodised cosinusoidal shape (Does et al., 2003; Parsons et al., 2003). For an apodised cosinusoidal MPG of dominant frequency f, an effective diffusion-time of $1/4f$ can be improvised by equating the *b*-values calculated for the PGSE and OGSE sequences. Under this prescription, OGSE has been used to observe diffusion-time dependent signal changes and subsequently estimate the surface-to-volume ratio in systems of packed beads (Parsons et al., 2003; Parsons et al., 2006). It has also been employed to investigate intracellular restriction effects in in vitro cell preparations (Colvin et al., 2011; Xu et al., 2011), and, most importantly, demonstrate diffusion-time dependent signal changes in normal and diseased rat brain in vivo (Colvin et al., 2008; Does et al., 2003). Even though it has recently been pointed out that the effective diffusion-time construct only applies over a limited range of frequencies (Novikov and Kiselev, 2011), it has nonetheless been firmly established that OGSE provides a unique image contrast and is therefore a promising technique for probing in vivo tissue microstructure.

In most previous studies with the OGSE sequence, little consideration has been given to the consequences of varying MPG orientation. If frequency-dependent effects are observable when the MPG is applied in one direction, it follows that combining orientation and frequency modulated experiments may provide an interesting new method to investigate tissue properties. A similar idea has already been considered for the PGSE and PGStE sequences, but in terms of the diffusion-time. For example, the diffusion-time dependence of the mean diffusivity (MD), which is the trace of the apparent diffusion tensor (ADT) divided by 3, has been investigated with those sequences in several studies (e.g. Le Bihan et al., 1993; Horsfield et al., 1996). In those cases, however, data was only acquired in the three principal gradient directions so that the full ADT and apparent anisotropy could not be estimated. The only study where the dependence of the full ADT on diffusion-time has been investigated was that by Clark et al. (2001), who found no changes to the ADT, possibly because the shortest diffusion-time employed in the study (8 ms) was too long. With the introduction of the OGSE sequence, it seems possible that alterations to the ADT with diffusion-time may be observable if the correspondence between MPG frequency and effective diffusion-time is utilised. The only earlier applications of this idea were to ex vivo monkey brain (Xu et al., 2010) and ex vivo mouse brain (Aggarwal et al., 2012). The goal of this study was to combine OGSE with DTI-like acquisition and analysis protocols to investigate the MPG frequency dependence of the ADT in rat brain in vivo. An interpretation of the results in terms of restricted diffusion and the effective diffusion-time is also considered. Some of the results have been previously presented in abstract form (Kershaw et al., 2010, 2011).

Materials and methods

All experiments were approved by the Animal Welfare Committee of the National Institute of Radiological Sciences, Chiba, Japan.

Eleven male Sprague–Dawley rats (age 7–9 weeks, weight 200–300 g) were anaesthetised with isoflurane (4% for induction and 2% during the imaging experiments) and fixed in a MRI compatible cradle with bite and ear bars. The cradle was inserted into the bore of the 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 (13 mm × 15 mm, Rapid Biomedical, Germany) was used for signal reception. The latter coil was positioned over the head of the animal centred near the interaural plane to maximise the signal received from both the cerebellum and caudal end of the cerebrum.

Data acquisition was performed with a four-shot SE-EPI sequence modified to have one apodised cosinusoidal MPG waveform placed on either side of the π-refocussing pulse (Does et al., 2003; Parsons et al., 2003; Colvin et al., 2008). The sequence was set up so that the user first selects the duration T of the MPG lobes, which determines the base frequency $f = 1/T$, and then the desired harmonics of f and the maximum *b*-value are entered. The *b*-value as a function of frequency is $(\gamma G_k/2\pi f_k)^2(1-1/8k)T$, where $f_k = kf$ is the frequency of the kth harmonic and G_k is the peak amplitude of the applied MPG (Does et al., 2003; Parsons et al., 2003). The code automatically set G_k so that b remains constant with increasing k. The code was also configured so that $b = 0$ data was acquired when a value of zero was included in the list of harmonics. After choosing the harmonics, the user selected the number of directions in which to apply the oscillating MPGs. Unit vectors defining the directions were either entered by hand or read in from a set of prepared files. Acquisition of the data for a single experiment then proceeded in two major loops: the outer loop cycled through the MPG directions and the inner loop cycled through the list of selected harmonics.

A 1 mm thick sagittal slice set at 1–1.5 mm away from the midplane of the brain was selected for imaging. This orientation and position ensured that the slice passed through both the cerebellum and the thickest part of the corpus callosum. Three experiments, distinguished by different sets of MPG parameters, were performed on each animal (see Table 1). Note that the selected harmonics were limited to those where G_k did not exceed 91% of the maximum available gradient (404 mT/m). Each experiment acquired images for 78 evenly distributed MPG directions. Other common imaging parameters were TR = 3 s, matrix size = 128 × 128, FOV = 25.6 mm × 25.6 mm, and spatial resolution = 0.2 mm × 0.2 mm × 1 mm. T_2-weighted anatomical images of the same slice were also acquired with a standard multi-SE sequence (TR = 3 s, 14 echoes, TE = 10–140 ms).

The data was reconstructed and analysed offline using purpose-written Matlab code. No spatial or temporal smoothing was applied at any point during the analysis. The six independent elements of the ADT were estimated pixel-by-pixel for each harmonic from each experiment on each animal using a standard DTI analysis technique (Basser et al., 1994). Partially guided by the MD and fractional anisotropy (FA) maps calculated from the lowest frequency data of the type A experiments listed in Table 1, regions-of-interest (ROIs) were drawn by hand on the anatomical images of each animal. The same ROIs

Table 1
Oscillating MPG parameters for the three experiments performed on each animal.

Expt	TE (ms)	T (ms)	Max b (s/mm^2)	k	f_k (Hz)
A	71	26	1000	1, 2	38.5, 76.9
B	71	26	450	1, 2, 3	38.5, 76.9, 115.4
C	79	30	400	1, 2, 3, 4	33.3, 66.6, 100, 133.3