Oscillating gradient diffusion MRI reveals frequency-dependent contrasts in cellular layers of the human cerebellum

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Target audience: Researchers who are interested in oscillating-gradient diffusion MRI contrasts and their microstructural basis in the human brain.

Purpose: We investigate the effect of gradient frequency on diffusion MR contrasts derived using oscillating diffusion-sensitizing gradients in the fixed human cerebellum. Water diffusion in nervous tissue is restricted by microscopic structural barriers such as cell membranes, intra- and extra-cellular structures. These restrictive effects form the microstructural basis of the temporal diffusion spectrum[1] in biological tissue. Oscillating-gradient spin echo (OGSE) acquisitions[2] allow sampling of the diffusion spectrum at high gradient frequencies (or ultra-short diffusion times), that are outside the regime of conventional pulsed-gradient diffusion MRI. The frequency-dependence of this diffusion spectrum can yield unique insights into local tissue microstructure and its spatial heterogeneity, as seen with recent contrasts in mouse brain[3]. The potential of OGSE acquisitions to generate tissue-specific or anatomically relevant contrasts in the human nervous tissue, however, remains to be investigated. Our findings here show, for the first time, generation of unique layer-specific contrasts with gradient frequency in cellular layers of the human cerebellum.

Methods: MR images of the human cerebellar lobes from two adult brains were acquired on an 11.7T Bruker spectrometer, using a diffusion-weighted spin echo sequence (TE/TR=37/1000 ms, 6 averages, 3 sagittal slices with slice thickness=1 mm). One set of diffusion experiments was performed using conventional pulsed diffusion-sensitizing gradients (0 Hz, effective diffusion time τ_{eff} of 15 ms), and four additional datasets were acquired with the diffusion-encoding module modified with oscillating gradient waveforms. Because of the short T2 of human cerebellar tissue (~31 ms), trapezoid-cosine diffusion gradients[4] were implemented in order to achieve maximal diffusion weighting within shorter gradient waveform durations. Data were acquired at four increasing gradient oscillation frequencies up to 200 Hz (τ_{eff} 1.25 ms). **Table 1** lists the diffusion parameters for OGSE experiments. At each frequency, one b0 image and 4 tetrahedrally-distributed diffusion directions (b ~700 s/mm^2) were acquired, at a resolution of 120 x 120 µm^2 in 1.5 hours. PGSE-based tensor data were acquired with six directions to compute direction-encoded color (DEC) maps. A voxel-wise linear least squares fitting of apparent diffusion coefficient (ADC) measurements versus gradient oscillation frequency was computed, to generate maps of the rate of change of ADC with frequency (denoted as Δ_fADC)[3]. The same specimens were then histologically processed for H&E staining, to allow direct comparison of the observed OGSE contrasts with histology.

f (Hz)	τ_{eff} (ms)	$G_{x,y,z}$ (mT/m)	b (s/mm^2)
67	3.73	173	700.2
100	2.5	316	700.3
150	1.67	400	700.6
200	1.25	499	700.0

Table 1: Trapezoid-cosine diffusion gradient parameters.

Results: Oscillating gradient diffusion MRI revealed distinct frequency-dependent contrasts in the human cerebellum. **Fig. 1** shows sagittal images of the cerebellum acquired using pulsed-gradient (f = 0 Hz) and oscillating-gradient (f = 200 Hz) sequences. The cerebellar cortex consists of an inner granular layer (CBGr) containing densely-packed granule cell bodies, and an outer molecular layer (CBml) that contains extensively branched dendrites and parallel fibers. The ADC maps acquired with PGSE and OGSE sequences revealed a marked difference in the relative contrast between these two cerebellar layers (**Fig. 1A-A'**). In the PGSE maps, the inner CBGr layer showed significantly ($p<0.005$) lower ADC compared to the outer CBml layer (Fig. 1A). At increasing gradient oscillation frequencies, this contrast was reversed, with the CBGr showing a drastic increase in ADC measurements with gradient frequency. As a result, ADC values in the CBGr were significantly ($p<0.001$) higher compared to the CBml at 150 Hz-200 Hz (Fig. 1A'). This contrast reversal between the two layers can be clearly seen from the comparison of ADC maps at 0 Hz and 200 Hz in **Fig. 1A-A'**. In comparison, the cerebellar white matter (indicated in the DEC map in Fig. 1B) showed no apparent changes in ADC with gradient frequency. Maps generated by a linear fitting of ADC versus frequency (Δ_fADC) clearly showed the strong contrast enhancement and specific delineation of the CBGr layer, which appeared significantly hyperintense compared to adjacent cerebellar tissue (**Fig. 1C**). Further, comparison of the resulting Δ_fADC maps with nuclei-specific H&E staining in the same specimens (**Fig. 2**) revealed that the highlighted regions corresponded specifically to the densely-stained CBGr layer in cerebellar folia. Quantitative ADC measurements (mean ± standard deviation) in the CBGr and CBml layers are shown in the plot in **Fig. 3**. The mean ADC in the CBGr layer was found to show a significant monotonic increase from 0.34 to 0.91 µm^2/ms (~2.7 times increase) between 0 to 200 Hz, and closely approximated a linear curve within this frequency range. The pulsed-gradient ADC values (at 0 Hz) were significantly lower in the CBGr than in the CBml. Mean ADC in the two layers approached approximately equal values at 67 Hz (0.56 ± 0.06 µm^2/ms in the CBGr versus 0.53 ± 0.04 µm^2/ms in the CBml), and the contrast continued to increase at higher oscillation frequencies. Comparison of the relative rates of increase in ADC clearly shows the reversed contrast with pulsed and oscillating-gradient measurements, and the progressive contrast enhancement with increasing frequency.

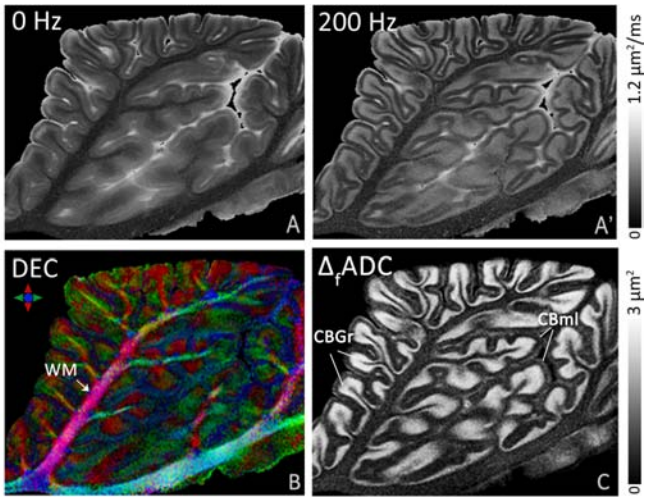

Fig 1: Frequency-dependent OGSE contrasts in the human cerebellum. A-A') Comparison of ADC maps at 0 Hz and 200 Hz shows reversed contrast between cerebellar granule (CBGr) and molecular (CBml) layers. DEC map of the cerebellar folia is shown in B. C) Linear fit of ADC versus frequency (Δ_fADC map) reveals the distinctly highlighted CBGr layer.

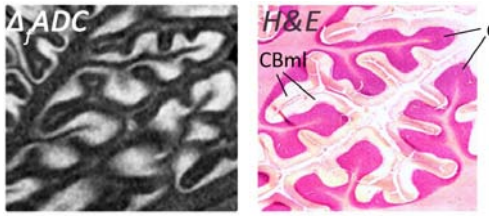

Fig 2: Δ_fADC map and H&E staining of the corresponding section.

Discussion & Conclusion: Our findings reveal the generation of unique layer-specific contrasts in cellular layers of the human cerebellum with high frequency OGSE acquisitions. The gradient-frequency dependence of ADC measurements in our study reflects underlying restriction to free water diffusion over spatial scales of ~2.6 to 9 µm (assuming a diffusion constant of 2.8 µm^2/ms at 37°C). The specificity of the ADC increase with frequency to the CBGr region indicates that OGSE contrasts over this frequency scale can sensitively probe the cytoarchitectural heterogeneity of cellular layers in the cerebellar gray matter. These findings demonstrate the potential of OGSE-based acquisitions to reveal microstructural contrasts in human cerebellar layers. Further, the examination of contrasts in the ex vivo cerebellum here allows direct correlation with histological staining *in situ*, which can provide insights into the microstructural basis of the diffusion spectrum in human nervous tissue.

Fig. 3

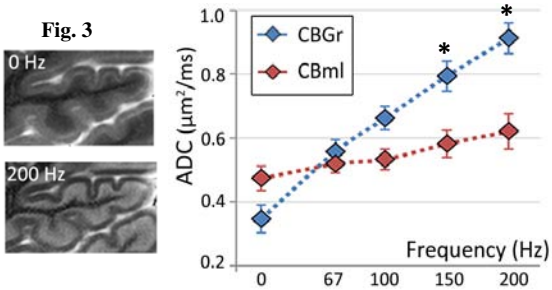

Fig 3 (Right): Relative contrast between CBGr and CBml cerebellar layers with oscillation frequency. The plot shows significant increase in ADC specific to the CBGr layer, leading to reversed ADC contrasts between the CBml (red) and CBGr (blue) layers with frequency. *indicates significantly ($p<0.001$) high ADC in CBGr compared to the CBml at 150-200 Hz.

References: [**1**] Gore *et al*, *NMR Biomed* 23, 2010 [**2**] Does *et al*, *Mag Res Med* 49, 2003 [**3**] Aggarwal *et al, Mag Res Med* 67, 2012 [**4**] Van et al, *Mag Res Med* 2013.
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