In vivo oscillating gradient diffusion MRI provides unique microstructural information in normal and hypoxic-ischemic injured mouse brains

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Target audience: Researchers who are interested in oscillating gradient diffusion MRI and its microstructural basis.

Purpose: Oscillating gradient spin-echo (OGSE) diffusion MRI is a unique tool to probe tissue microstructures at varying length scales [1]. The dependence of ADC measurements on oscillating frequency has been observed in animal [2,3] and human brains [4,5]. New OGSE-based tissue contrasts that highlight specific neuronal structures in the cerebellum and hippocampus have been demonstrated in post-mortem tissue specimens [6,7], but similar tissue contrasts have not been reported *in vivo*. In this study, we compared *in vivo* and *ex vivo* OGSE measurements in normal and injured mouse brains to investigate the microstructural basis of the OGSE-based tissue contrasts and their applications.

Methods: *In vivo* OGSE experiments were performed on an 11.7 T horizontal spectrometer using cosine-trapezoid oscillating gradient waveforms [5] and a four-segment EPI readout. For the adult mouse cerebellum (n=5), pulsed gradient spin echo (PGSE, δ/Δ = 4/20 ms) and OGSE (100 - 200 Hz) data were acquired using a cryo-genic probe with TE/TR = 44/2000 ms, NA=8, 10 diffusion directions, b = 500 s/mm^2, resolution = 0.1mm x 0.1mm x 0.6mm. For neonatal mice (P11, n=5) at 24 hours after unilateral hypoxic-ischemic injury [8], PGSE and OGSE (50 - 200 Hz) data were acquired using a 10 mm planer surface coil and 72 mm quadrature transmitter coil with TE/TR = 52/2000 ms, NA=4, 6 diffusion directions, b = 600 s/mm^2, resolution = 0.2mm x 0.2mm x 0.8mm. The *in vivo* ADC values were calibrated based on signals from an agorose gel phantom placed beside the mouse brain. Δ_fADC was calculated by linear fitting of the ADC values with oscillating frequency. The animals were perfused fixed with 4% PFA. *Ex vivo* experiments were performed on a 17.6 T vertical spectrometer with similar parameters as *in vivo* scans but higher b-values, and sample temperature set at 37 ^{o}C.

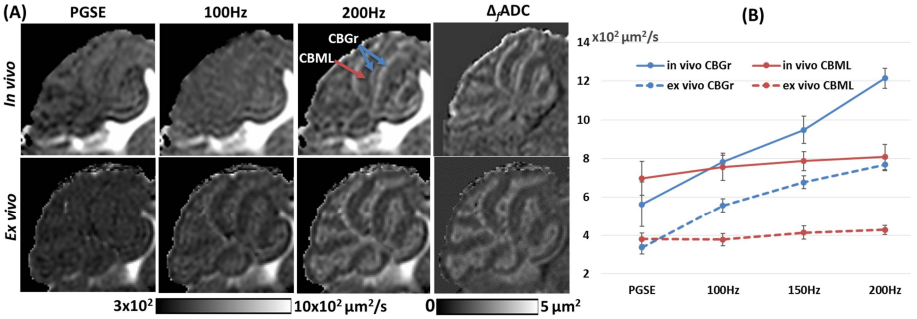

Fig. 1 (A): *In vivo* and *ex vivo* ADC maps of the mouse cerebellum acquired using PGSE and OGSE sequences and corresponding Δ_fADC maps. (B): *In vivo* (solid) and *ex vivo* (dash) ADC values in the granule cell layer (CBGr, blue) and molecular layer (CBML, red).

Results: *In vivo* OGSE measurements of the mouse cerebellum differed from *ex vivo* in two aspects: *i*) *in vivo* ADC values were significantly higher than *ex vivo* ADC values at 50-200 Hz; *ii*) The rates at which the *in vivo* ADC values increase with frequency (Δ_fADC) were different from *ex vivo*, which resulted in different frequency-dependent tissue contrast (Fig. 1A). For example, *in vivo* Δ_fADC of the CBGr was 3.46 ± 0.21 μm^2, significantly higher than *ex vivo* 2.21 ± 0.20 μm^2. The degree of changes was structure dependent both *in vivo* to *ex vivo* (CBGr *v.s.* CBML in Fig. 1B). Pathology could also change OGSE-based tissue contrasts. Among the five neonatal mice, one showed severe cytotoxic edema, with reduced PGSE-ADC values and hyper-intense T_2 signals in the ipsilateral cortex, hippocampus, and thalamus than the contralateral side (Fig. 2A). As frequency increases, the OGSE ADC maps showed less clearly defined edema regions compared to the PGSE results (Fig. 2A). OGSE-ADC measurements in the edema region increased faster with frequency than the contralateral side, and the ADC values from the cortical edema region became undistinguishable from the contralateral side at 200 Hz (Fig. 2B). In comparison, the difference in *ex vivo* ADC measurement between the edema and contralateral regions were reduced.

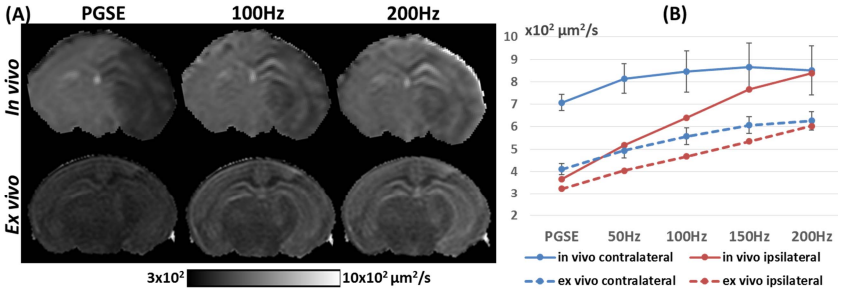

Fig. 2 (A): ADC maps of a P11 mouse brain after HI injury using PGSE and OGSE, *in vivo* and after perfusion fixation. (B): *In vivo* (solid) and *ex vivo* (dash) PGSE and OGSE ADC values in the contralateral (blue, n=5) and ipsilateral (red) cortex.

Discussions and conclusions: *In vivo* OGSE experiments are challenging due to the long TE required to accommodate oscillating gradient waveforms and limited maximum b values. Using the trapezoid oscillation gradient waveforms and high-sensitivity coils, we improved the contrast-to-noise ratio such that the neuronal layers can be clearly defined from surrounding tissues in the live mouse brain. *In vivo* and *ex vivo* tissues differed in absolute ADC values, frequency dependency (Δ_fADC), as well as their contrasts in the PGSE/OGSE ADC maps. The reduced ADC and slightly lower Δ_fADC in the *ex vivo* tissues may be the results of changes in membrane permeability and cell dimension after death and chemical fixation. The increase in Δ_fADC after cytotoxic edema may be caused by cell swelling, and its disappearance after chemical fixation may be the results of changes in membrane permeability. The observation that OGSE ADC measurements at 200 Hz showed less tissue contrasts for cytotoxic edema than PGSE measurements suggests that the microstructural changes in this case were within certain spatial scale (> 5 μm) . More extensive studies will be needed to investigate the relationships between OGSE signals and pathology in this model.

References: 1) NMR Biomed 2010 23(7):745-56. 2) MRM 2003 49(2):206-15. 3) Neuroimage 2013 70:10-20. 4) MRM 2013 doi: 10.1002/mrm.24632. 5) MRM 2013 doi:10.1002/mrm.24987. 6) MRM 2012 67(1):98-109. 7) ISMRM 2013 p.2073. 8) Dev Neurosci 2005 27:81-86.