

Imaging neuronal degeneration in the mouse hippocampus after neonatal hypoxia-ischemia using oscillating gradient diffusion MRI

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Introduction: Hypoxia-ischemia (HI) in neonatal mouse models results in significant grey and white matter damages. The hippocampus exhibits selective neuronal vulnerability as early as 24 h following HI, with progressive neuronal degeneration over time [1]. While structural T₂-weighted MRI and diffusion tensor MRI are sensitive to subsequent edema and white matter injury respectively, the detection of early neuronal degeneration after HI remains challenging. Recent reports have shown that oscillating gradient diffusion MRI (dMRI) can generate grey matter contrasts that are sensitive to the spatial heterogeneity of cellular microstructures, by varying the modulation frequency of the diffusion-sensitizing gradients [2,3]. Specifically, frequency-dependent contrasts with this technique can highlight pyramidal and granule cell layers in the mouse hippocampus, regions that are selectively vulnerable to HI. In this study, we hypothesize that microstructural changes due to degeneration of pyramidal and granule cells in the neonatal hippocampus after HI can be detected using oscillating gradient dMRI. Our results demonstrate, for the first time, that frequency-dependent contrasts with this technique can detect and delineate progressive neuronal degeneration in the mouse hippocampus following HI.

Methods: Postnatal day 7 (P7) C57Bl/6 mice were subjected to unilateral HI using the Vannucci model, and euthanized at 1, 4 and 8 days after HI (P8, P11 and P15, with n=4 at each stage). Age-matched non-injured mice (n=4 at each stage) were used as controls. 3D ex vivo images of the brains were acquired on an 11.7T spectrometer using a diffusion-weighted gradient and spin echo (DW-GRASE) sequence with navigator phase correction previously developed by our group [3] (N_{eff} = 4, TE/TR = 54/800 ms, NA=4, diffusion gradient duration = 20 ms), with the diffusion-encoding module consisting of conventional pulsed gradients (0 Hz, effective diffusion time Δ_{eff} of 15 ms), and cosine-modulated oscillating gradients at three different oscillation frequencies of 50, 100 and 150 Hz (Δ_{eff} of 5, 2.5 and 1.67 ms, respectively). At each frequency, diffusion-weighted images in four tetrahedral directions (b-value ~700 s/mm²) and one non diffusion-weighted (b0) image were acquired at a resolution of 100 x 100 x 100 μ m³ and scan time of ~1.2 h. A Linear least squares fitting of the measured apparent diffusion coefficient (ADC) with the gradient oscillation frequency was computed, to generate maps representing the rate of change of ADC with frequency (denoted as Δ_f ADC). Group-averaged Δ_f ADC maps of control and HI-injured brains at each age were generated by nonlinear deformation of the images to age-matched reference brain templates using diffeomorphic metric mapping.

Results & Discussion: Oscillating gradient dMRI revealed frequency-dependent contrasts in the neonatal mouse hippocampus. Comparison of Δ_f ADC contrast at P8 with nissl-stained histology (Fig. 1A,A') showed that regions highlighted in the Δ_f ADC maps corresponded to the pyramidal cell layer (Py) in the CA1-CA3 hippocampal regions and the granule cell layer in the dentate gyrus (GrDG). As shown in [3], both these layers consist of populations of densely-packed neuronal cell bodies. Fig. 1 (A,B) shows a comparison of group-averaged Δ_f ADC maps of the control and HI-injured brains at day 1 after injury (P8). The Δ_f ADC values in the ipsilateral Py layer ($1.37 \pm 0.21 \mu$ m²) in HI-injured brains were significantly lower compared to values measured in the contralateral region ($2.34 \pm 0.06 \mu$ m², n=4, p<0.005). Staining of the ipsilateral hippocampus with fluoro-jade, a marker of neurodegeneration, revealed a large number of degenerating neurons in the Py layer at this stage (white arrows in Fig. 1B'). Plots of ADC versus gradient oscillation frequency in the Py layer (Fig. 1C) showed increasing differences between the ipsilateral and contralateral regions at higher frequencies, whereas no significant differences could be observed in ADC measurements with conventional pulsed-gradient dMRI (0 Hz in Fig. 1C). At P8, the GrDG region showed no evidence of neurodegeneration in the fluoro-jade stained sections (orange arrows in Fig. 1B'), and in comparison, no significant differences between the ipsilateral and contralateral Δ_f ADC values were observed in the GrDG layer at this stage. These findings suggest that frequency-dependent Δ_f ADC contrasts are uniquely sensitive to early neuronal degeneration in the hippocampus immediately after HI injury.

Further, oscillating gradient dMRI at 1, 4 and 8 days following HI revealed the spatiotemporal evolution of neurodegeneration in the hippocampus (Fig. 2). Similar to the observations at P8, Δ_f ADC values in the ipsilateral Py layer were significantly lower compared to control and contralateral regions progressively till P15 (Fig. 2, white arrows). In comparison, differences to a less degree in Δ_f ADC in the ipsilateral and contralateral GrDG layers were first observed 4 days after HI, and progressed thereafter (Fig. 2, orange arrows). These temporal changes in Δ_f ADC are consistent with histological evidence of delayed and less severe neurodegeneration in the dentate gyrus following HI in the neonatal mouse [1].

The results of the current study illustrate that oscillating gradient dMRI can detect neurodegeneration-induced changes at microstructural scales in the mouse hippocampus following HI, which are difficult to discern with conventional pulsed gradient dMRI, and suggest that the frequency-dependent Δ_f ADC contrast with this technique can be used as a novel indicator of early neuronal degeneration in the mouse HI model.

References: [1] Sheldon *et al*, *Neurosc Letters* 304, 2001 [2] Gore *et al*, *NMR in Biomed* 23, 2010 [3] Aggarwal *et al*, *Mag Res Med* 2011 (doi: 10.1002/mrm.22981)

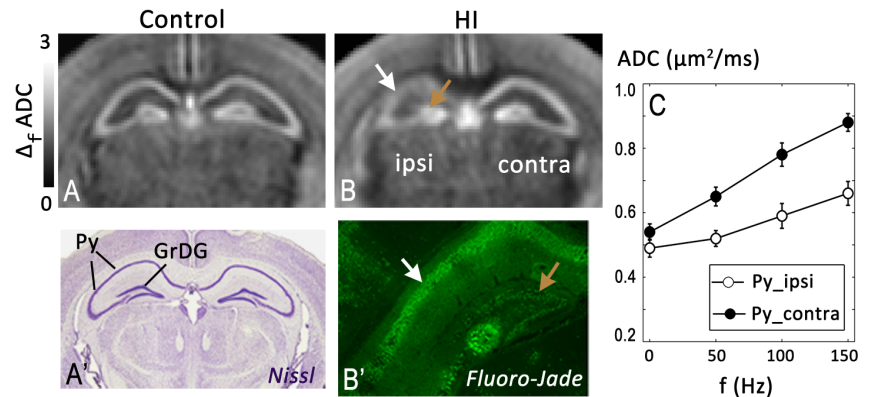


Fig. 1: Frequency-dependent dMRI contrasts in the P8 mouse hippocampus. A-A') Comparison of Δ_f ADC with nissl-stained histology shows enhancement of the pyramidal cell layer (Py) and the granular layer of the dentate gyrus (GrDG). B-B') Decrease in Δ_f ADC in the ipsilateral Py after HI (white arrows) correlates with neurodegeneration in the fluoro-jade stained section. C) Measured ADC as a function of frequency in the ipsi- and contralateral Py after HI-injury.

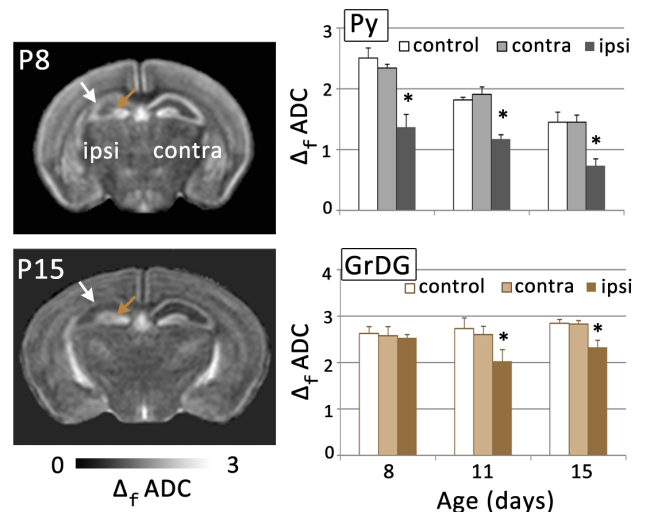


Fig. 2: Temporal evolution of Δ_f ADC in the mouse hippocampus following neonatal HI. Left panel) Group-averaged Δ_f ADC maps of HI-injured brains at P8 and P15 showing the Py (white arrows) and GrDG (orange arrows) layers. Right panel) Δ_f ADC measurements in the control, contralateral and ipsilateral Py (Top) and GrDG (Bottom) layers at 1, 4 and 8 days post-HI, revealing the spatiotemporal patterns of HI-induced progressive neuronal degeneration (* p<0.005).