Acute visual function impairment in EAE is primarily caused by optic nerve inflammation as assessed by DBSI
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Introduction
Experimental autoimmune encephalomyelitis (EAE) is the main animal model of human multiple sclerosis. Optic neuritis is a commonly seen pathology in EAE mice, and involves inflammation, demyelination, and axonal damage. A prior histological study showed that inflammation precedes retinal ganglion cell death in EAE. [1] Diffusion tensor imaging (DTI) has previously detected axon and myelin injury as decreased axial and increased radial diffusivity, respectively, in chronic EAE optic nerve [2]. In this study, a novel diffusion basis spectrum imaging (DBSI) [3] was performed to evaluate inflamed EAE-affected optic nerves during acute EAE.

Materials and Methods
Animal Model: EAE was induced in female, 8 – 10 week old C57BL/6 mice (~20g) by active immunization with MOG35-55 peptide/CFA emulsion (n=15). In vivo diffusion MRI was immediately performed at the first detection of impaired vision in mice, typically between 9 – 14 days after immunization. Age-matched control mice (n=5) underwent the same procedure without MOG35-55 immunization. In vivo MRI: DBSI was performed on a 4.7 T magnet utilizing a multi-echo spin-echo diffusion-weighted sequence by multi-directions and multi-b-values. All images were obtained with acquisition parameters of TR 1.5 sec, TE 37 ms, Δ 18 ms, δ 6 ms, max. b-value 2,200 s/mm², slice thickness 0.8 mm, field-of-view 22.5 mm × 22.5 mm, in-plain resolution 117 μm × 117 μm (59 μm × 59 μm zero filled). Functional measurement: Visual acuity (VA) of the individual mouse eye was assessed using Virtual Optomotor System (OptoMotry, Cerebral Mechanics Inc., Canada). Independent left-eye and right-eye vision was measured corresponding to clockwise and counter-clockwise grating movement with adjustable spatial frequency [4]. Baseline VA measurement was assessed to confirm intact vision before EAE immunization. Daily VA measurement was performed to determine EAE optic neuritis onset. Normal mice VA = 0.38 ± 0.03 c /d (mean ± SD). Poor mouse vision was defined as VA ≤ 0.25 c/d.

Results
Figure 1(A) shows diffusion-weighted image of control mouse brain with left and right optic nerve (see highlighted box). Figure 1(B) shows representative DBSI maps of cell %, edema %, and inflammation % (equal to cell % + edema %) on control and acute EAE mice with decreased VA. Cell inflammation, edema, and inflammation were clearly seen in acute EAE compared to control mice. Quantified DBSI index and DTI-derived axial and radial diffusivity were shown in Figure 2 and 3. Figure 2 shows all three DBSI index linearly correlates with VA. A strong negative correlation of edema with VA [R² = 0.64; Figure 2(b)] and inflammation with VA [R² = 0.80; Figure 2(c)] was seen in acute EAE mice. Figure 3(a) shows that DTI- and DBSI-derived axial diffusivity in EAE mice were each significantly decreased compared with control mice. In contrast, Figure 3(b) shows that DTI- and DBSI-derived radial diffusivity in EAE each significantly increased compared with control mice.

Discussion and Conclusion
Our results indicate that in vivo DBSI-derived inflammation% significantly correlated with VA during acute EAE, supporting a predominant role of inflammation at the onset of optic neuritis. The decreased axial diffusivity measured by both DTI and DBSI suggested accompanying axonal injury during acute EAE. Immunohistochemistry is being performed on nerves fixed after MRI to evaluate cell infiltration, axonal injury, and myelin integrity to compare and correlate with our in vivo MRI findings.

References

Fig. 1 A representative in vivo transverse view diffusion-weighted image of a control mouse brain with optic nerves highlighted (A). Representative DBSI maps of control and EAE optic nerve demonstrating the extent of optic nerve inflammation are presented with color-coded intensity (B).

Fig. 2 Correlations between optic nerve DBSI indices and visual acuity (VA) of control (n=5) and acute EAE mice with various degrees of VA impairments (n=15). (a) Cell %, (b) edema %, and (c) inflammation % all negatively correlated with mouse VA. DBSI-based inflammation in optic nerves correlated strongly (R² = 0.80) with mouse VA, suggesting inflammation is a predominant factor leading to visual function impairment in acute EAE.

Fig. 3 (a) DTI- and DBSI- derived axial diffusivities in acute EAE decreased by 20% and 15% from the control level. (b) DTI- and DBSI- derived radial diffusivities in acute EAE increased by 80% and 38% from the control level. *, p < 0.05 compared to control.