

# Effects of Chronic Ocular Hypertension on Microstructural Integrity of the Visual System using Diffusion Tensor MRI

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**Target Audience:** Researchers and clinicians with interest in basic and translational applications of diffusion tensor imaging (DTI) to pathophysiology and vision neuroscience.

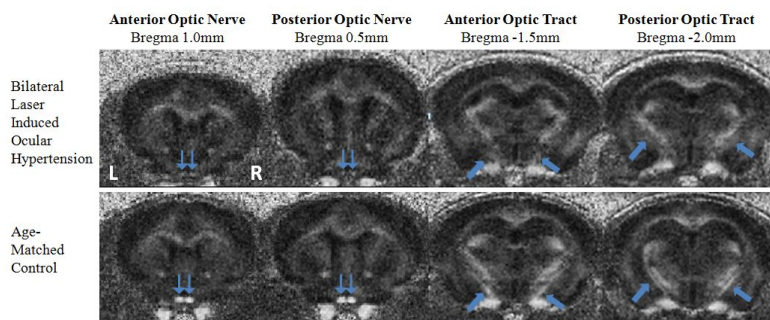
**Purpose:** Glaucoma is the second leading cause of blindness in the world [1] and is a major public health problem in the elderly. While elevated eye pressure is a major risk factor, it has been shown recently that glaucoma may also involve the brain [2]. However, the neurodegenerative mechanisms of glaucoma in the visual system are still poorly understood. DTI has been suggested to reflect the microstructural integrity of the visual pathways in animal models of acute retinal ischemia and optic nerve injury [3, 4]. In this study, we evaluated the feasibility and sensitivity of DTI to detect mild, chronic microstructural changes spatially along the optic nerve and optic tract in a mouse model of laser-induced ocular hypertension mimicking chronic glaucoma in humans to better understand this disease in the brain.

**Methods:** **Animal Preparation:** The intraocular pressure (IOP) of 7 adult C57BL/6 mice was increased by disrupting the balance of aqueous outflow in both eyes using 532nm green laser photocoagulation to the trabecular meshwork [5]. IOP was measured using a Tonolab rebound tonometer regularly before and after laser damage, and was shown to be significantly elevated for more than 6 months, with IOP at  $28.1 \pm 6.6$  mmHg at 6 months after laser treatment compared to baseline IOP at  $14.2 \pm 6.5$  mmHg ( $p < 0.01$ ). DTI was performed to monitor the microstructural integrity of the visual pathway in the late stage at about 8 months after laser treatment. Six age-matched mice were untreated and served as control. Mice were anesthetized with a mixture of air and isoflurane (3% for induction and 1.25% for maintenance) during MRI experiments. **MRI Protocols:** All scans were performed using a 9.4-Tesla/31-cm Varian/Agilent horizontal bore scanner with a 32mm transmit-receive volume coil. DTI were acquired using a fast spin echo sequence, with 12 diffusion gradient directions at  $b = 1.0 \text{ ms}/\mu\text{m}^2$  and two additional B0 images at  $b = 0 \text{ ms}/\mu\text{m}^2$ . Other imaging parameters included: TR/TE = 2300/27.8ms, ETL = 8,  $\delta/\Delta = 5/17 \text{ ms}$ , NEX = 4, FOV =  $2.0 \times 2.0 \text{ cm}^2$ , acquisition matrix =  $192 \times 192$  (zero-filled to  $256 \times 256$ ), and slice thickness = 0.5mm. Slices were oriented orthogonal to the prechiasmatic optic nerves. **Data Analysis:** Co-registration between B0 and diffusion-weighted images were performed using SPM8. DTI parametric maps including fractional anisotropy (FA), axial diffusivity ( $\lambda_{||}$ ), radial diffusivity ( $\lambda_{\perp}$ ) and mean diffusivity (MD) maps were computed using DTIStudio. Manual regions of interest were drawn on anterior/posterior optic nerves at Bregma 1.0/0.5mm and anterior/posterior optic tracts at Bregma -1.5/-2.0mm respectively based on FA,  $\lambda_{||}$  and  $\lambda_{\perp}$  maps to minimize cerebrospinal fluid contamination. DTI parametric values along the visual pathway were compared between experimental and control groups using two-tailed unpaired t-tests. Results were considered significant when  $p < 0.05$ .

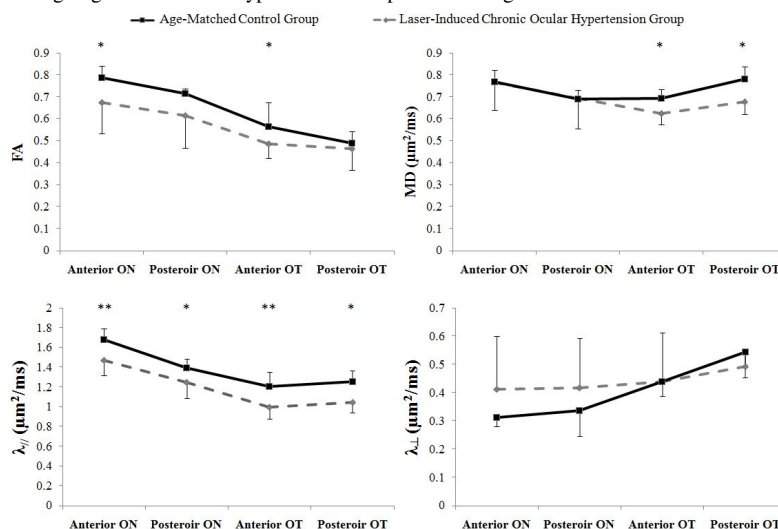
**Results:** Qualitative comparisons in Figure 1 show that the optic nerves and optic tracts undergoing chronic ocular hypertension had a lower FA compared to the age-matched control. Quantitatively, there was a significantly lower FA and  $\lambda_{||}$  along the anterior and posterior optic nerves and optic tracts undergoing chronic ocular hypertension ( $p < 0.05$ ) (Figure 2).  $\lambda_{\perp}$  was higher in the experimental optic nerves with marginal significance ( $p = 0.14$ ). No obvious  $\lambda_{\perp}$  difference was found in the optic tracts between groups. Differences in  $\lambda_{||}$  between groups were fairly constant in the entire visual pathway measured. However, the differences in FA and  $\lambda_{\perp}$  were smaller toward the posterior optic tract. Significantly lower MD was observed in the optic tracts but not the optic nerves in the experimental group.

**Discussion and Conclusion:** Ocular hypertension may induce optic neuropathy along the visual pathway [6, 7]. Based on FA differences between groups, it is apparent that chronic ocular hypertension led to more severe microstructural disorganization in the anterior portions (optic nerve) relative to the posterior portions (optic tract) of the visual pathway. This appears to indicate Wallerian-like anterograde degeneration as a possible candidate of the disease mechanisms of glaucoma in the brain [8]. It has been shown that  $\lambda_{||}$  and  $\lambda_{\perp}$  were preferentially sensitive to axonal and myelin integrity respectively [9]. Whether the significantly lower  $\lambda_{||}$  along the entire visual pathway measured and the marginally significant  $\lambda_{\perp}$  increase in the optic nerve in the experimental group indicated axonal damage and demyelination remains to be elucidated via histological confirmation in future studies. Once confirmed, our observations of fairly constant  $\lambda_{||}$  difference along the visual pathway and obvious  $\lambda_{\perp}$  difference in the optic nerves but not optic tracts may also suggest differential progressive rates of different neurodegenerative events along the visual pathway under chronic ocular hypertension similar to a recent DTI study on acute retinal ischemia [3]. The significantly lower MD in the optic tracts but not the optic nerves in the experimental group appears to be attributed to the significantly lower  $\lambda_{||}$  but not  $\lambda_{\perp}$  in the optic tract. In conclusion, DTI of laser-induced chronic ocular hypertensive mice provided a non-invasive model to assess the effects of risk factors of glaucoma on the microstructural changes in the visual pathway, and may provide insights on the mechanisms of disease progression in human glaucoma for devising better strategies for neuroprotection.

**References:** [1] Kingman S., et al. Bull World Health Organ. 2004; [2] Alm A., et al. Br J Ophthalmol, 2006; [3] Sun S., et al. Neuroimage, 2008; [4] Zhang X., et al. Glaucoma, 2011; [5] Du Y. et al. ARVO, 2013; [6] Morrison J.C., et al. J Glaucoma 2005; [7] Gazzard G., et al. J Ophthalmol 2003; [8] Saggau S., et al. BMC Neurosci 2010; [9] Beaulieu C., et al. NMR in Biomed, 2002



**Figure 1.** Fraction anisotropy (FA) maps of a representative laser-induced ocular hypertensive mouse and an age-matched control at the level of anterior/posterior optic nerves at Bregma 1.0/0.5mm to anterior/posterior optic tracts at Bregma -1.5/-2.0mm respectively. Hypointensity was observed in the optic nerves and tracts (blue arrows) undergoing chronic ocular hypertension compared to the age-matched control.



**Figure 2.** DTI quantitation from anterior/posterior optic nerve (ON) to anterior/posterior optic tract (OT) in the chronic ocular hypertension group and age-matched control group (two-tailed unpaired t-tests between experimental and control groups: \* $p < 0.05$ ).