Longitudinal characterization of brain microstructure and visuomotor behavior following acute ocular hypertension using diffusion tensor imaging, magnetization transfer imaging and optokinetics

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Target Audience: Researchers and clinicians interested in MR neuroimaging and optokinetic applications to help determine eye-brain-behavior relationships and pathophysiological mechanisms related to vision-related diseases such as retinal ischemia and glaucoma.

Purpose: Glaucoma is a term used for a collection of neurodegenerative diseases that cause damage to the visual pathway and irreversible vision loss over time. Acute intraocular pressure (IOP) elevation can be found in angle-closure glaucoma patients, and is often associated with retinal ischemia and other cardiovascular diseases including stroke. At the same time, not every subject with ocular hypertension (OHT) will develop glaucoma throughout their lifetime. This leads to a need to understand how different etiology of IOP elevation may affect the structural integrity, metabolic activity and ultimately the functional outcomes in the visual system as these diseases progress. In this study, we used an experimental rat model of acute OHT to introduce an episode of elevated IOP to two different severities (mild and severe) in order to probe the effects on the structure and function of the visual system spatiotemporally with MR neuroimaging and optokinetics. By determining the changes in microstructural integrity and visual behavioral response in the injured visual system our results may help understand the disease mechanisms associated with OHT and may provide an in vivo model system to assess targeted preventive and treatment strategies.

Methods: ***Animal Preparation:*** Sixteen adult Long Evans rats received unilateral OHT in the right eye at 40mmHg (mild; n=9) or 90mmHg (severe; n=7) for 60 minutes via physiological saline anterior chamber perfusion under ketamine/xylazine anaesthesia [1]. The left eye was untreated and served as an internal control ***MRI Protocols:*** Animals were scanned at 3, 7 and 14 days after OHT using a 9.4-Tesla/31-cm Varian/Agilent scanner with a volume transmit and surface receive coil. Diffusion tensor imaging (DTI) was acquired using a fast spin echo sequence, with 12 diffusion gradient directions at b=1.0ms/μm^2 and 2 non-diffusion-weighted images at b=0ms/μm^2 (b_0). Other imaging parameters included: TR/TE= 2300/27.8ms, ETL=8, δ/Δ=5/17ms, NEX=4, FOV=2.6x2.6cm^2, acquisition matrix= 192x192 (zero-filled to 256×256), and slice thickness=0.5mm. Slices were oriented orthogonal to the prechiasmatic optic nerves. In addition, magnetization transfer imaging (MTI) was acquired at the level of the prechiasmatic optic nerves to a subgroup of 2 mild and 3 severe OHT animals, with 9.5μT saturation pulses at 4000Hz off-resonance. ***MRI Data Analysis:*** DTI paramatric maps including fractional anisotropy (FA), axial diffusivity ($\lambda_{//}$) and radial diffusivity ($\lambda_{\perp}$) maps were computed using DTIStudio. Regions of interest were manually drawn on the prechiasmatic optic nerve (ON) and the optic tract (OT) based on FA, $\lambda_{//}$, and $\lambda_{\perp}$ maps and the rat brain atlas. DTI parametric values were compared between experimental and control visual pathways of each group using two-tailed paired t-tests. Magnetization transfer ratio for the optic nerve was calculated as MTR = $(M_0-M_T)/M_0$ ***Optokinetics:*** OptoMotry virtual reality system (CerebralMechanics, Inc.) was used to assess the visuomotor behavior by quantifying the visual acuity (VA) of each eye before, and at 7 and 14 days after OHT induction [2-3]. Spatial frequency ranged from 0.042 to 0.750 cycles/degree using a simple staircase method while rotation speed (0.12 degrees/s) and 100% contrast were kept constant.

Results: In both mild and severe groups, DTI showed significantly lower FA and higher $\lambda_{\perp}$ in the right ON and left OT projected from the injured eye compared to the uninjured visual pathways from the opposite eye throughout the experimental period (Figs. 1 and 2). Significant positive correlation was observed between MTR and FA of the optic nerve at day 7 (r=0.81; p<0.05) and day 14 (r=0.72; p<0.05) but not day 3 (r=0.40; p>0.05) (Fig. 3). Significant negative correlation between MTR and ($\lambda_{\perp}$) was also observed at day 7 (r=-0.79; p<0.05). In the optokinetic analysis in Fig. 4, the VA before OHT was comparable between both eyes and VA of the uninjured eye remained unchanged across time (p>0.05). Both mild (40mmHg) and severe (90mmHg) OHT groups had significantly decreased VA at days 7 and 14 (two-tailed paired t-tests, p<0.05). In addition, the right injured eye showed significantly worse VA after severe OHT than mild OHT at both days 7 and 14 (two-tailed unpaired t-tests, *p<0.05). No significant difference in visuomotor behavior was found between days 7 and 14 within mild or severe groups (two-tailed paired t-tests, p>0.05).

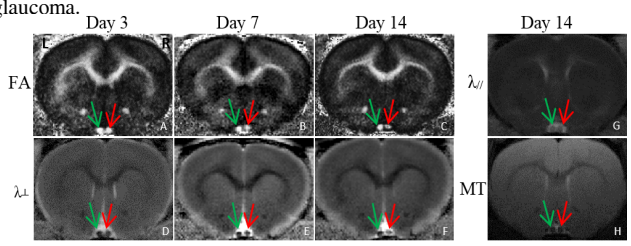

Figure 1: (First 3 columns) Fractional anisotropy (A-C) and radial diffusivity (D-F) maps of the uninjured (green arrows) and injured (red arrows) prechiasmatic optic nerves at 3, 7, and 14 days after ocular hypertension (OHT) at 90mmHg for 60 min. (Last column) Axial diffusivity map (G) and magnetization transfer contrast image (H) at 14 days after OHT at 90mmHg for 60 min

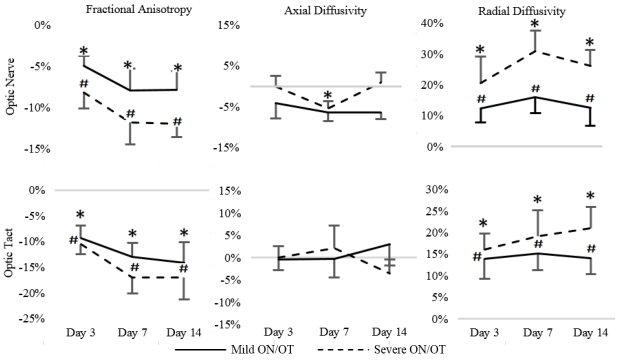

Figure 2: Percentage changes of fractional anisotropy (left), axial diffusivity (middle) and radial diffusivity (right) in injured optic nerve and optic tract relative to the opposite uninjured visual pathways at 3, 7, and 14 days after mild or severe OHT (two-tailed paired t-tests between left and right visual pathways, mild: *p<0.05; severe: #p<0.05)

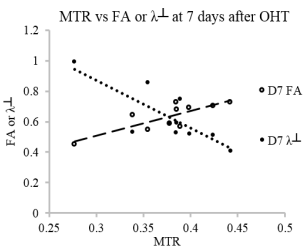

Figure 3: Scatter plots of MTR vs FA or $\lambda_{\perp}$ in the optic nerve at 7 days after OHT

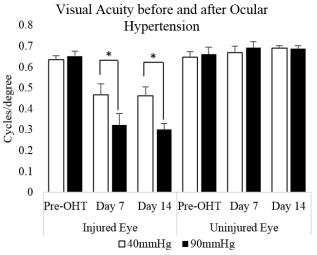

Figure 4: Visual acuity (VA) before, and at 7 and 14 days after OHT. (two-tailed unpaired t-tests, *p<0.001

Discussion and Conclusions: Our results indicated that acute OHT at 40 and 90mmHg for 60 min led to significant changes in FA and $\lambda_{\perp}$, but not $\lambda_{//}$ along the visual pathway throughout the experimental period. In addition, MTR in optic nerve appeared to be reduced and strongly correlated with FA and $\lambda_{\perp}$, but not $\lambda_{//}$. These results appeared to be different from previous reports stating that changes in $\lambda_{//}$ preceded changes in $\lambda_{\perp}$ after severe acute ocular hypertension that involved transient retinal ischemia [4-5]. Possible explanations to this discrepancy may include difference in species used (rats vs mice) as well as a lower IOP induced in our study compared to previous studies at 100-120mmHg which could potentially cause more damages and higher sensitivity to detect $\lambda_{//}$ changes. Our preliminary Mn-enhanced MRI study at 5 weeks after acute OHT from the same batch of animals used in this study also showed no significant alterations in anterograde Mn transport between injured and uninjured visual pathways (n=5, unpublished), indicative of relatively preserved axonal integrity over time. These findings shed light on the different predominant neurodegenerative events occurring after varying degrees of OHT, and the need to target treatment with reference to past IOP history in patients. The longitudinal profiles of overall microstructural integrity (as reflected by FA) in the visual system appeared to correspond with the visuomotor behavioral changes observed at 7 and 14 days after both mild and severe OHT. Further studies will compare a wider range of IOP elevation to probe the corresponding pathophysiological events and the corresponding visual outcomes more precisely with MR neuroimaging, optokinetics and histological confirmation.

References: [1] Li S., Int J Ophthalmol 2010; [2] Prusky GT., IOVS, 2004 [3] Douglas RM., Vis Neurosci, 2005; [4] Sun SW., NeuroImage, 2008; [5] Sun SW., NeuroImage, 2006