Spatiotemporal Characterization of Neurodegeneration in the Visual System upon Acute and Chronic Optic Neuropathies 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: To determine spatiotemporally the progression of microstructural disorganization in acute and chronic optic neuropathies of different severity using DTI; to better understand the mechanisms of pathological processes in glutamate excitotoxicity, and in neurodegenerative diseases such as ocular hypertension and glaucoma.

Methods: ***Animal Preparation:*** Fourteen adult Sprague-Dawley rats were divided into 2 groups. Six rats were intravitreally injected with 5μL of 40mM N-methyl-D-aspartate (NMDA) in the right eye to induce acute glutamate excitotoxicity to the retina (NMDA group; acute, severe injury). Eight rats received an injection of a mixture of 5μL of 10μm microbeads (MB) and 5μL of 15μm microbeads (MB group; chronic, mild injury) into the anterior chamber of the right eye to block aqueous outflow and induce sustained elevated intraocular pressure (IOP) [3] up to experimental period at about 4 weeks after MB injection. The left eye was untreated and served as an internal control. IOP was measured in both eyes for the MB group using the Tonolab rebound tonometer regularly after MB injection, and was found to be 32±12 mmHg in right, treated eye and 15±5 mmHg in left, control eye at about 2-3 weeks after MB injection. Rats were anesthetized with a mixture of air and isoflurane (3% for induction and 1.5% for maintenance) during MRI experiment at Day 3, Week 1, Week 2 and Week 4 after NMDA injection, and at Week 1 and Week 4 after MB injection. ***MRI Protocols:*** All scans were performed using a 9.4-Tesla/31-cm Varian/Agilent horizontal bore scanner with a volume transmit and surface receive coil. DTI was acquired using a fast spin echo sequence, with 12 diffusion gradient directions at b=1.0ms/μm^2 and two b=0ms/μm^2 (B0). 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. ***Data Analysis:*** Co-registration between B0 and diffusion-weighted images were performed using SPM8. DTI paramatric 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 nerve at Bregma 2.5/1.5mm, and optic tract at Bregma -3.5mm based on FA, $\lambda_{//}$,$\lambda_{\perp}$ maps to minimize cerebrospinal fluid comtamination. DTI parametric values were compared between experimental and control visual pathways of each group using two-tailed paired t-tests.

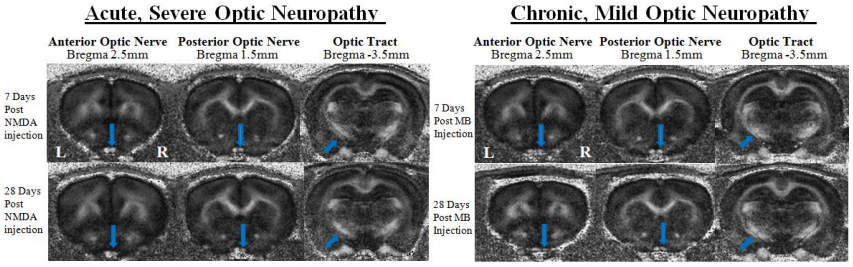
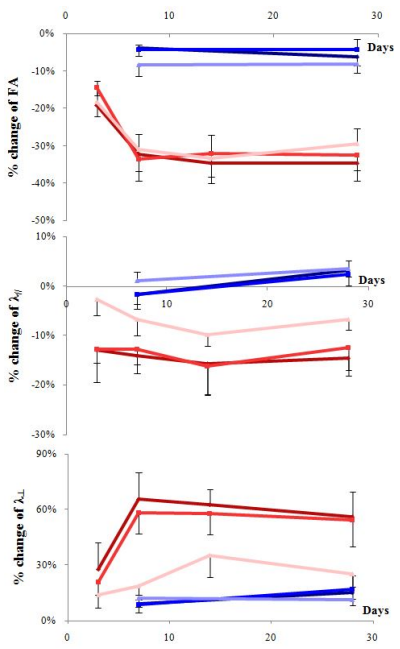


Figure 1. Fraction anisotropy (FA) maps of NMDA-induced acute retinal injury (left) and microbead (MB)-induced chronic ocular hypertension (right) at 7 and 28 days post-injection at the levels of anterior/posterior optic nerve (at Bregma 2.5/1.5mm), and optic tract (at Bregma -3.5mm). Blue arrows indicate degenerating visual pathway projected from right, experimental eye.



	Day 3	Week 1	Week 2	Week 4
FA	***	***	***	***
	***	***	***	*
	*	***	***	***
	N/A	n.s.	N/A	*
	N/A	**	N/A	n.s.
	N/A	*	N/A	**
$\lambda_{//}$	*	**	*	***
	**	**	*	*
	n.s.	*	***	*
	N/A	n.s.	N/A	*
	N/A	n.s.	N/A	n.s.
	N/A	n.s.	N/A	*
$\lambda_{\perp}$	n.s.	***	***	***
	*	***	***	***
	n.s.	***	*	***
	N/A	n.s.	N/A	**
	N/A	*	N/A	n.s.
	N/A	*	N/A	**

Figure 2. (Left) Percentage change of DTI parameters along visual pathway projected from right, injured eye relative to left, untreated eye in NMDA group (red) and MB group (blue) at 3 days to 4 weeks after injury; (Right) Result table of two-tailed paired t-tests between treated and untreated visual pathways (*p<0.05; **p<0.01; *** p<0.001; n.s.: not significant; N/A: data not available).

Results: Qualitative comparisons in Figure 1 show an apparently decreasing FA in right optic nerve and left optic tract (blue arrows) upon NMDA-induced retinal injury or MB-induced ocular hypertension to the right eye. DTI quantitation in Figure 2 showed a significant decrease in FA along optic nerve to optic tract projected from right eye in NMDA group throughout experimental period (red lines/asterisks). Larger $\lambda_{//}$ decrease and $\lambda_{\perp}$ increase were also observed in optic nerve compared to optic tract. The degenerative patterns in FA and $\lambda_{\perp}$ progressed in optic nerve from post-injection Day 3 to Day 7 and retained afterwards, whereas for optic tract, $\lambda_{//}$ and $\lambda_{\perp}$ possessed a delayed decrease and increase respectively at Day 7 which progressed until Day 14. For MB group, there was a significant decrease in FA and increase in $\lambda_{\perp}$ in the posterior visual pathway with no significant difference in $\lambda_{//}$ at Day 7. The DTI parametric changes progressed spatiotemporally to Day 28 with a small but significant increase in $\lambda_{//}$ (Figure 2 blue lines/asterisks).

Discussion and Conclusion: NMDA injection has been shown to result in massive glutamate excitotoxic retinal and optic nerve damage [2], whereas microbead-induced ocular hypertension may induce milder, chronic degeneration along the visual pathway [3]. Based on the differences in $\lambda_{//}$ and $\lambda_{\perp}$ changes between optic nerve and optic tract, it is apparent that NMDA-induced excitotoxic injury led to severe microstructural disorganization more pronounced in the anterior visual pathway than the posterior.. This suggests Wallerian-like anterograde degeneration as a potential neurodegenerative mechanism in glutamate excitotoxicity. $\lambda_{//}$ and $\lambda_{\perp}$ have been reported to be sensitive to axonal and myelin integrity respectively [4]. Our observations of delayed $\lambda_{\perp}$ increase relative to early $\lambda_{//}$ decrease along the NMDA-injured visual pathway may also suggest differential progressive rates of neurodegenerative events such as axonal damage and demyelination along the visual pathway under glutamate excitotoxicity, similar to a recent DTI study on acute retinal ischemia [5]. For MB-induced ocular hypertension, it is currently unclear why the posterior visual pathway possessed more significant DTI parametric changes relative to the anterior visual pathway at Day 7. One possible explanation may be related to the early distal-to-proximal axonopathy recently demonstrated in a similar animal model of glaucomatous neurodegeneration [3]. Further confirmation is currently ongoing to elucidate this issue. Future studies will also combine optical coherence tomography and MRI to correlate the neurodegenerative events between retina and optic pathway longitudinally in the same animals.

References [1] Morrison J.C., J Glaucoma 2005; [2] Lam T.T., Inves Ophthalmol Vis Sci., 1999; [3] Crish S. D.,PNAS, 2010; [4] Beaulieu C., NMR in Biomed, 2002; [5] Sun S.W., Neuroimage, 2008;