Differentiation of Primary and Secondary Degeneration in the Visual Pathway using in vivo Mn-enhanced MRI

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INTRODUCTION: Traumatic injury to the central nervous system is accompanied by the spreading damage of secondary degeneration, which results in further loss of neurons and function away from the initial injury (1). After partial optic nerve (ON) injury, intact retinal ganglion cells (RGCs) and axons undergo secondary damages, but the topographic distributions and degenerative events are still unclear (2). In this study, we explore the capability of 3D Mn-enhanced MRI (MEMRI) for in vivo, longitudinal evaluation of primary and secondary degeneration along the retinocollicular projections after partial ON injury in rats.

MATERIALS AND METHODS: Animal Preparation: The superior region of the right intraorbital ON in 10 adult Sprague-Dawley rats (200-250 g) was partially transected at about 2 mm from the eye. One week and 6 weeks after surgery, $MnCl_2$ solution (3μL, 50mM) was injected intravitreally into both eyes of the same animals. MEMRI was performed 1 day after Mn^{2+} administration. Throughout the experiment, the left ON was not transected and the visual pathway projected from the left eye to the right visual brain served as an internal control. **MRI Protocol:** All MRI measurements were acquired utilizing the 7 T Bruker scanner using a receive-only surface coil. 3D spin-echo T_1-weighted (T1W) imaging was acquired covering the entire visual pathway, with TR/TE = 250/6.7ms, FOV = 32x32x16mm^3, acquisition resolution = 200x200x200μm^3, RARE factor = 4 and number of averages = 4. Total scan time was 40 mins. **Data Analysis:** Maximum intensity projection (MIP) was performed onto the 3D T1W images after segmenting the visual pathway from the retina to the subcortex in both hemispheres. Histograms of the distributions of T1W signal intensities (SI) were obtained in the lateral and medial halves of the left and right superior colliculi (SC) using ImageJ v1.44p at the same volumes (lateral/medial SC: 45%/55% of whole SC), and were normalized to the mean T1W SI in the right medial SC of each animal. The averages of normalized T1W SI in the lateral and medial SC were compared intra- and inter-hemispherically, and between Week 1 and Week 6 using two-tailed paired t-tests. T1W SI in the vitreous, retina and ON were also compared between contralateral hemispheres using two-tailed paired Student's t-tests. Results were considered significant when $p<0.05$. **Histology:** After MRI experiments at Week 6, 4 animals were randomly selected for histological verification of the location and severity of injury in the partially transected ON at about 1.5 mm posterior to the eye using toluidine blue staining.

RESULTS: In the MIP images in Fig. 1, intravitreal Mn^{2+} injection into the left control eye resulted in robust T1W enhancements in the visual pathway from the left retina to the superficial layers of the contralateral right SC and the right lateral geniculate nucleus, whereas in the contralateral right eye at both 1 week and 6 weeks after partial ON transection (arrowhead in Fig. 1), the right vitreous and retina, and the right ON distal to the transection site possessed significantly higher and lower T1W SI respectively than the contralateral hemisphere ($p<0.05$). In the posterior visual brain, localized T1W hypointensity was observed in the left lateral SC [open arrows in Fig. 1 (middle and bottom)], with a clear border separating the lateral and medial (closed arrows in Fig. 1) halves of left SC. Histograms in Fig. 2 indicated a shift in the distributions of T1W SI in both left lateral and medial SC toward lower SI compared to the right SC at both Week 1 and Week 6, with the left lateral SC shifting more than the left medial SC [Fig. 2 (top and middle)]. Interestingly, a slight shift in the distribution of T1W SI was also observed in the left medial SC toward lower SI from Week 1 to Week 6 [Fig. 2 (bottom)]. Quantitative analyses showed that the left lateral SC had about 28%±5% and 27%±3% reductions in T1W SI at Week 1 and Week 6 respectively compared to the right SC ($p<0.001$), whereas the left medial SC had lower T1W SI by 11±6% and 16±5% compared to the right SC at Week 1 and Week 6 respectively ($p<0.001$). The T1W SI of left medial SC was significantly higher at Week 1 than at Week 6 ($p<0.01$), and was significantly higher than that of the left lateral SC at both time points ($p<0.001$). No statistical significance was found when comparing between right lateral and medial SC at the same time points, or in the left lateral SC, or right lateral and medial SC between Week 1 and Week 6 ($p>0.05$). Histology in Fig. 3 confirmed the presence of degeneration in both superior and inferior ON, with the superior ON being more severely injured as indicated by more pronounced myelin debris formation and atrophy.

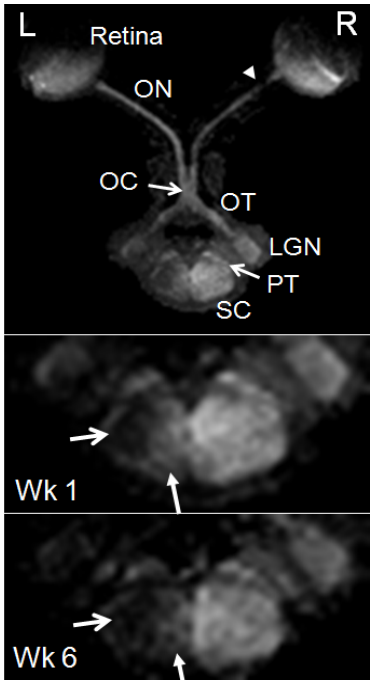

Fig. 1: Axial MIP of the visual pathway from retina to visual brain at 1 week (top and middle) and 6 weeks (bottom) after partial transection of the right superior ON and at 1 day after intravitreal Mn^{2+} injection. (OC: optic chiasm; OT: optic tract; LGN: lateral geniculate nucleus; PT: pretectum)

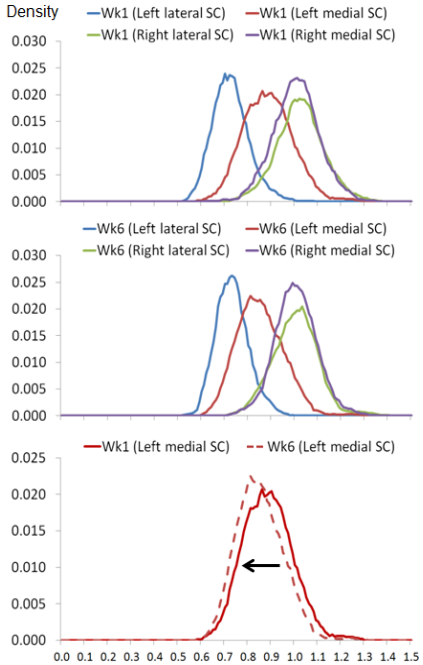

Fig. 2: Histograms of the distributions of T1W SI in the lateral and medial halves of left and right SC in all rats 1 week (top) and 6 weeks (middle) after partial transection of right superior ON. (Bottom) A slight but significant shift in the distribution of T1W SI was observed in the left medial SC toward lower SI from Week 1 to Week 6. (X-axis: normalized T1W SI)

DISCUSSIONS AND CONCLUSION: The results of this study demonstrated the feasibility of in vivo, high-resolution MEMRI for assessing the primary and secondary degeneration topologically and longitudinally along the visual pathways. Previous histological studies showed that partial transection of the superior intraorbital ON led to rapid loss of directly injured RGCs in superior retina at 1 week, and delayed secondary loss of RGCs in inferior retina at 4 weeks after injury (3). Since the RGC axons emanating from superior retina projected to lateral SC of the contralateral hemisphere in rodents (4), the consistent T1W hypointensity in left lateral SC at Week 1 and Week 6 appeared to reflect the primary loss of topological connections and Mn^{2+} transport in retinocollicular projections. The slightly weaker T1W enhancement in left medial SC at Week 1 might be partly due to reduced Mn^{2+} transport upon spreading of oxidative stress through the inferior retina early after partial ON injury (5,6), whereas the further T1W SI reduction in left medial SC at Week 6 might indicate secondary loss of RGCs and axons projecting through the uninjured, inferior ON (3,6). The current MEMRI results open up new opportunities for longitudinal assessments of secondary changes along uninjured neural connections in various neurodegenerative diseases and injuries and upon therapeutic interventions in future studies.

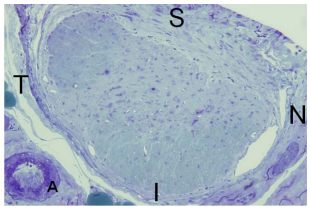

Fig. 3: Light micrograph of the intraorbital ON section at about 1.5 mm behind the injured eye at Week 6. (toluidine blue stain, 200×)(S: superior; I: inferior; T: temporal; N: nasal; A: ophthalmic artery).

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