# Cornea damage did not affect topical-loaded Manganese-Enhanced MRI

Tiffany Thiel[1], Endre Agoston[1], Keelan Tuel[1], Ravi Rajpoot[1], Hsiao-Fang Liang[1], and Shu-Wei Sun[1,2]
[1]*Loma Linda University, Loma Linda, CA, United States,* [2]*University of California, Riverside, CA, United States*

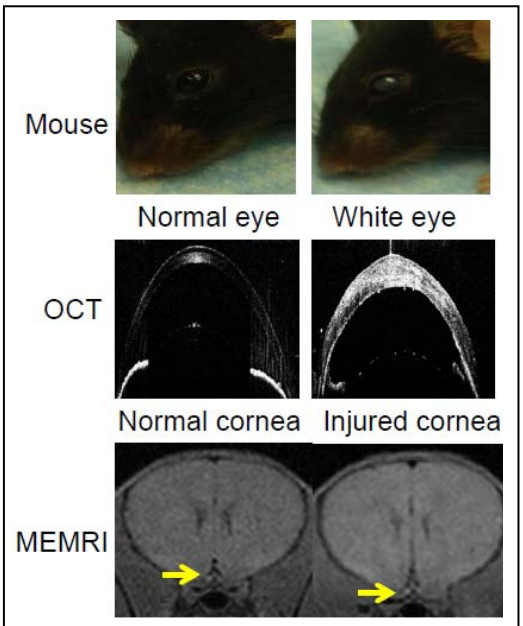


**Figure 1. OCT and MEMRI of the normal-eye and white-eye mice.** 3 of 23 mice showed white eyes at the end of MEMRI time courses. OCT confirmed that these animals had opaque corneas. Interestingly, the MEMRI of the visual system remained normal in these animals.

## Introduction

$Mn^{2+}$ Enhanced MRI (MEMRI) is a powerful tool to evaluate the retinal ganglion cells (RGCs) and their axonal conductivity (1-3). We previously demonstrated the feasibility of using a topical loading approach as an alternative approach of the traditional intravitreal injection to provide Mn2+ solution for MEMRI in the mouse visual system (3). A topical drop of 1 M $MnCl_2$ led to signal enhancement from retina to superior colliculus in a day and decayed to the undetectable levels in 1 week. Topical loaded MEMRI enhanced the feasibility of applying MEMRI in a time course study. In this project, we performed biweekly 1M topical loaded MEMRI on mice for 14 weeks. At the end of MEMRI time course, Optic Coherence Tomography (OCT) was used to detect cornea and retina layers. Diffusion Tensor Imaging (DTI) was used to examine the optic nerve integrity as an index of the retinal damage.

## Materials and Methods

23 healthy mice (8-week-old female C57BL/6) were anesthetized by 1.2% isoflurane/oxygen. Body temperature was maintained using an electric heating pad. For the topical administration, 5 µl 1.0M $MnCl_2$ was provided to the surface of the right eye on each mouse. After one hour, the remaining solution was carefully removed by lint-free tissue (Kimwipes, Ontario, Canada). At 24 hours after administration of $MnCl_2$, mice were anesthetized for imaging. T1WI was taken using a Bruker 4.7T BioSpec animal scanner with TR of 250 ms, TE of 8 ms, FOV of 1.5cm, and data matrix of 128 x 128 (with zero-padding to 256 x 256). The topical loaded MEMRI was conducted biweekly for 14 weeks (~ 3 months). At the end of time courses, anterior segment and retina OCT was performed (Bioptigen Inc, Research Triangle Park, NC) with 250 A-scans per B-scan, 250 B-scan frames, and 1024 samplings/A-scan in depth. DTI was performed to examine the optic nerve integrity with TR 3 s, TE 29 ms, diffusion gradient pair ($\Delta$) = 20 ms, diffusion gradient duration ($\delta$) = 3 ms, a six-direction diffusion scheme with b-values of 0 and 0.85 ms/µm$^2$. Relative anisotropy (RA), Axial Diffusivity (AD), Radial Diffusivity (RD), and Trace (TR) were quantified from normal and MEMRI-affected nerves.

## Results

Four of 23 mice showed abnormal eyes at the end of MEMRI time courses. Three mice showed white eyes, and one mouse has closed eye. Using OCT, the white eyes were confirmed to have opaque corneas (**Fig, 1**). Among the tested 23 mice, one mouse was found to have the eye closed by eyelids. DTI showed that this eye was associated with damage to optic nerves with a reduced RA, reduced AD, and increased RD (**Fig. 2**). Except the closed-eye mouse, all 22 mice showed MEMRI signal increments similar to one measured from the normal mice (**Fig. 3**).

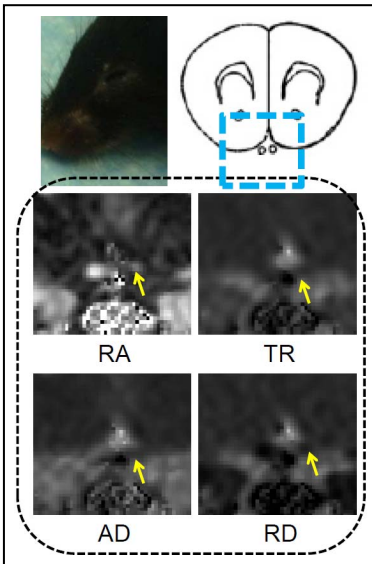


**Figure 2.**
**DTI of the closed-eye mouse.** DTI confirmed the optic nerve damage as decreased RA, decreased AD, and increased RD (arrows indicated), suggesting RGC damage caused by the repeated MEMRI experiments.

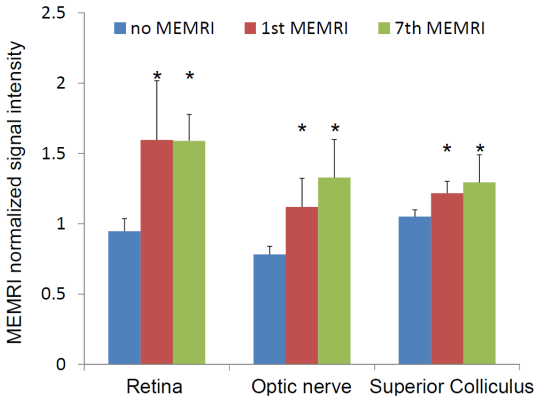


**Figure 3. The 1st and the 7th (last) MEMRI.** *: $p < 0.05$, compared to the no-MEMRI signals.

## Discussion and Conclusions

Three of 23 tested mice showed opaque corneas and one mouse showed optic nerve damage induced by repeated performances of topical loaded MEMRI. Although the abnormal cornea did not affect the visual system to upload Mn2+ from retina up to superior colliculus, such cornea damage may be an early sign of damage to the posterior ocular damage, the retinal damage. Although topical loading approach minimizes the invasive process to conduct visual MEMRI, repeated MEMRI may be harmful to the visual system.

## References

(1) Pautler RG, NMR Biomed 2004;17:595-601. (2) Lindsey JD, Neuroimage 2007;34:1619-1626. (3) Sun et al, IOVS, 2011; 52: 3914–3920.

## Acknowledgement

NIH R01 NS062830.