In vivo MEMRI of Neuronal Plasticity in Retinocollicular Projection

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INTRODUCTION: The retinocollicular projection between retina and the superficial layers of superior colliculus (SC) in rodents is a commonly used model system for evaluating the mechanisms of retinotopic map formation, neurodegeneration and plasticity in the brain (1). Despite the increasing number of studies investigating retinotopic projections in visual brain development and disorders (1), *in vivo*, high-resolution imaging of the plastic changes in retinotopic organization in the subcortical brain nuclei has not yet been available. Mn^{2+} has been increasingly used as a T_1-weighted contrast agent for neuronal tract tracing (2) and functional brain mapping at lamina levels (3). In this study, we explore the capability of high-resolution Mn-enhanced MRI (MEMRI) for *in vivo* assessment of the reorganization of retinocollicular projections in rat models of early postnatal visual impairments.

MATERIALS AND METHODS: Animal Preparation: Sprague-Dawley rats (N=18) were prepared and divided into 3 groups. In Group 1 (n=6), neonatal monoculear enucleation (ME) was performed to the right eyes at postnatal day (P) 1. In Group 2 (n=6), monocular deprivation (MD) was performed by suturing the eyelids of the right eyes at the time of eyelid opening at P15. Six other animals were untreated and acted as a control (CTRL). At 8 weeks old, $MnCl_2$ solution (2μL, 100mM) was injected intravitreally into the left eyes of all animals. MEMRI was performed 1 day after Mn^{2+} administration. **MRI Protocol:** All MRI measurements were acquired utilizing the 7 T Bruker scanner using a receive-only surface coil. 2D spin-echo T_1-weighted (T1W) imaging was acquired at an oblique orientation parallel to the superficial layers of the SC, with TR/TE = 400/7.5ms, FOV = 32x32mm^2, MTX=200x200, slice thickness = 0.5mm, no. of slices = 9, RARE factor = 4 and number of averages = 60. Total scan time was 15 mins. Before Mn^{2+} injection, diffusion tensor imaging (DTI) was also acquired to complement the MEMRI findings with the structural integrity along the visual pathways, using 4-shot SE-EPI diffusion weighted images with FOV = 32x32 mm^2, MTX = 128 x 128, slice thickness = 1 mm, number of slices = 15, TR/TE = 3750/30 ms, b = 0 and 1000 s/mm^2 and 30 diffusion directions. **Data Analysis:** T1W signal intensities (SI) in the superficial layers of SC in each hemisphere were measured from each group using ImageJ v1.42q, and were normalized to a non-visual area in the brain. For DTI, fractional anisotropy (FA) maps were obtained using DTIStudio v2.30 after co-registration. FA values along the major visual pathways projected from the left eye [left prechiasmatic optic nerve (L-PON), and right anterior (R-AOT) and posterior optic tract (R-POT)] and from the right eye [right prechiasmatic optic nerve (R-PON), and left anterior (L-AOT) and posterior optic tract (L-POT)] were measured using ImageJ. T1WSI in SC, and FA values in each PON, AOT and POT in the same hemispheres were compared among ME, MD and CTRL using two-tailed unpaired t-tests. Results were considered significant when $p<0.05$.

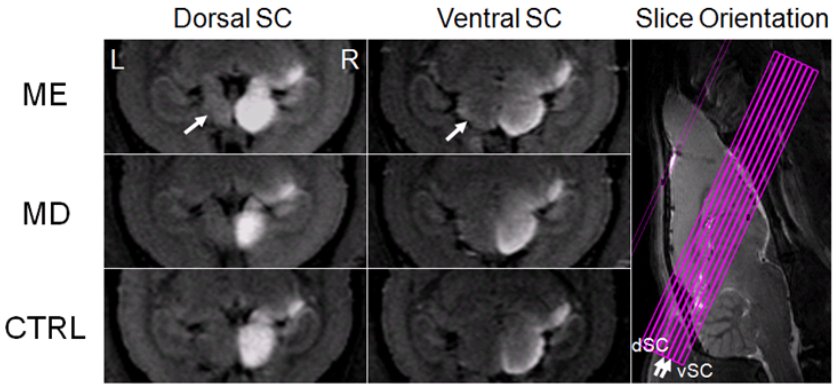

Fig. 1: 2D T1-weighted images (T1WI) of the superior colliculus (SC) at 1 day after Mn^{2+} intravitreal injection into the left eyes of the monocularly enucleated (ME), monocularly deprived (MD) and control animals. Robust Mn enhancement could be observed in the entire right dorsal SC (dSC, left column), and at the border of the right ventral SC (vSC, right column) in all groups. Note also the mild enhancement in the superficial layers of left SC in the ME group (arrows). Right column indicates the slice orientation of oblique 2D T1WIs in the sagittal plane.

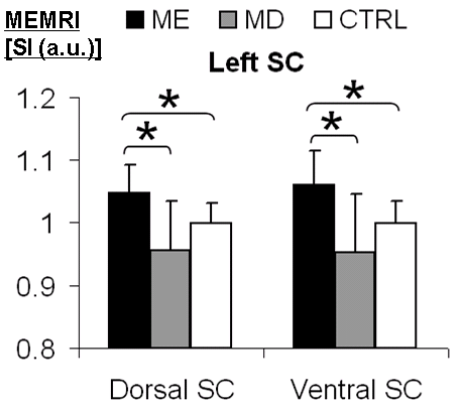

Fig. 2: Comparisons of T1W SI of the superficial layers of left SC in the entire dorsal SC, and the border of ventral SC in the left brain (Two-tailed unpaired t-test, *p<0.05).

RESULTS: In the T1W images in Fig. 1, intravitreal Mn^{2+} injection into the left eye resulted in enhancements in the entire superficial layers of the contralateral right SC in all animals. Mild enhancement could also be observed in the ipsilateral left SC in the ME group (arrows), but was not apparent in MD or CTRL group. Quantitative evaluations in Fig. 2 indicated a significantly higher T1W SI in the superficial layers of ipsilateral left SC in the ME animals than MD and CTRL animals ($p<0.05$). No significant SI difference was observed in right SC among ME, MD and CTRL ($p>0.05$). For DTI (Fig. 3), a significantly lower FA was found in the ME rats along the entire visual pathway projected from the enucleated right eye (right), and in the L-POT of MD rats. Interestingly, a significantly higher FA was found in the L-PON of the ME rats compared to both MD and CTRL rats.

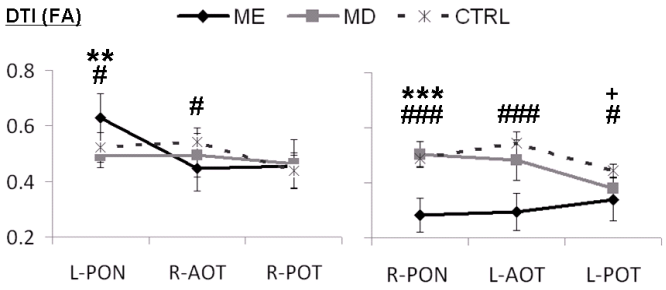

Fig. 3: Comparisons of FA values along the visual pathways projected from the left (left) and right eyes (right). (Two-tailed unpaired t-test between ME and MD, *p<0.05, **p<0.01, ***p<0.001; Two-tailed unpaired t-test between ME and CTRL, #p<0.05, ##p<0.01, ###p<0.001; Two-tailed unpaired t-test between MD and CTRL, +p<0.05, ++p<0.01, +++p<0.001)

DISCUSSIONS AND CONCLUSION: In DTI, the lower FA in R-PON, L-AOT and L-POT of ME rats and L-POT of MD rats likely suggested neurodegeneration of the afferent fibers projected from the enucleated/deprived eye (4). On the other hand, the comparable Mn enhancements in right SC among animal groups were likely ascribed to the higher integrity of crossed optic fibers (R-AOT and R-POT) from the left, untreated eye. Previous histological studies showed that neonatal unilateral eye removal (ME) but not visual deprivation (MD) alone resulted in a significant increase in the number of aberrant, ipsilaterally projecting retinal ganglion cells in the remaining eye compared to normal rats (4-6). The results of this study demonstrated the sensitivity of MEMRI in detecting neural plasticity of the uncrossed retinal projections in left SC in vivo after neonatal ME. Whether the higher FA in L-PON of ME rats was related to the retention of optic nerve axons from the ipsilaterally projecting retinal ganglion cells of the left eye (4) remained to be elucidated. Future MEMRI studies are envisioned that measure the development and reorganization of uncrossed retinal projections in disease, plasticity, drug interventions and genetic modifications in a global and longitudinal setting.

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