In vivo MEMRI of Early Postnatal Development in Rat Visual System

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INTRODUCTION: The retinocollicular and retinogeniculate projections in rodents are a well-established model system for evaluating the mechanisms of retinotopic map formation, neurodegeneration and plasticity during early postnatal visual development (1). However, to date, no tools have yet been available for in vivo, high-resolution investigations of retinotopic projections globally along the neonatal visual pathways. Mn^{2+} has been increasingly used as a T_1-weighted contrast agent for in vivo neuronal tract tracing (2) and functional brain mapping at lamina levels in the adult brains (3). In this study, we explore the capability of high-resolution Mn-enhanced MRI (MEMRI) for in vivo assessment of retinal projections in the early postnatal rat brains before natural eyelid opening, and compare the Mn^{2+} enhancements between neonatal and adult visual brain nuclei after intravitreal Mn^{2+} injection into one eye.

MATERIALS AND METHODS: Animal Preparation: Sprague-Dawley (SD) rats (N=40) and C57BL/6J mice (N=10) were divided into 5 groups. At postnatal days (P) 1, 5, 10 and 60, 6 SD rats from each age group were injected intravitreally with 100mM $MnCl_2$ solution into the right eye. MEMRI was performed 8 hours (Hr8) and 1 day (D1) after Mn^{2+} administration. Considering the increasing vitreal volume and decreasing retinal ganglion cell and optic nerve counts in rats with age (4,5), the Mn^{2+} injection volume was chosen to be 0.5, 1.0, 1.5 and 2.0 μL respectively at P1, P5, P10 and P60. This resulted in vitreal concentrations (vit. conc.) below toxic levels shown to inhibit terminal field enhancement in both adult rat and mouse superior colliculi (SC) (6,7). Given the similar vitreal volumes and brain sizes between adult mice and neonatal rats at P1 (4), MEMRI was also performed to 6 P60 mice after intravitreal Mn^{2+} injection into the right eye at the same dosage as P1 rats. Four age-matched rats/mice without Mn^{2+} injection were scanned from each age group as a control (CTRL). **MRI Protocol:** All MRI measurements were acquired utilizing the 7 T Bruker scanner using a mouse brain volume coil for neonatal SD rats and adult P60 mice, and a receive-only surface coil for adult P60 SD rats. 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 = 20x20mm^2 for neonatal rats and adult mice and 32x32mm^2 for adult rats, MTX=200x200, slice thickness = 0.5mm, no. of slices = 9, RARE factor = 4 and number of averages = 60. Total scan time was 15 mins. **Data Analysis:** Maximum intensity projection (MIP) was performed onto the 2D T1W images covering the dorsal and ventral SC for qualitative evaluation of Mn-enhancement in the posterior visual brain. Signal intensities (SI) in SC and lateral geniculate nuclei (LGN) of each hemisphere were measured using ImageJ v1.43u, and were normalized to a non-visual area in the brain. Mn^{2+} enhancement was quantified by calculating the rate of signal increase at Hr8 and D1 compared to CTRL animals. Values of each brain nuclei in the same age groups were compared using two-tailed unpaired or paired t-tests. Results were considered significant when $p<0.05$.

RESULTS: In the MIP images in Fig. 1, intravitreal Mn^{2+} injection into the right eye resulted in T1W hyperintensity consistently in the contralateral left SC and LGN at Hr8 in all age groups. Such enhancement was also observed at D1 in the adult rats and mice at P60, but was less obvious in the neonatal rats at P1, P5 and P10. Mild Mn^{2+} enhancement could also be found in the ipsilateral right visual components particularly in the neonatal rat SC at Hr8; In the quantitative analyses in Fig. 2, significant increase in T1W SI was found in the contralateral left SC and LGN in all neonatal and adult brains at Hr8. Interestingly, while the SI of left SC and LGN continued to increase in the P60 rats ($p<0.05$) at D1, or remained the same in the P60 mice ($p>0.05$), the SI of left SC and LGN dropped significantly in the neonatal rats at D1 compared to Hr8. In the right SC, a small but significant increase in SI could be observed in the neonatal brains at Hr8, as well as the adult mice at D1. In contrast, little SI changes could be observed in the right LGN.

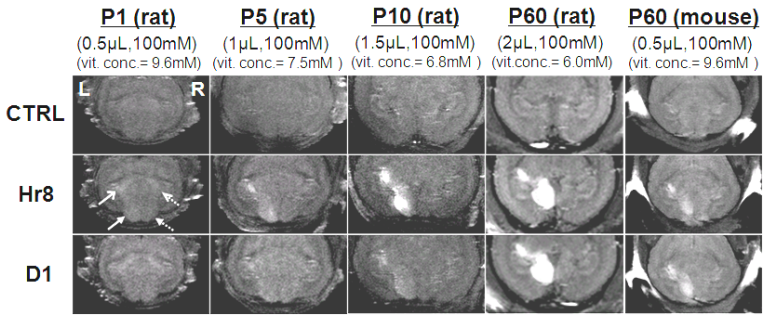

Fig. 1: (a) MIP images of the posterior visual brain nuclei at P1, P5, P10 and P60 in rats, and at P60 in mice with (Hr8 and D1) and without (CTRL) Mn^{2+} injection. Regions of interests were drawn in the left SC (solid arrow), left LGN (solid, open arrow), right SC (dashed arrow) and right LGN (dashed open arrow) for quantitative analyses.

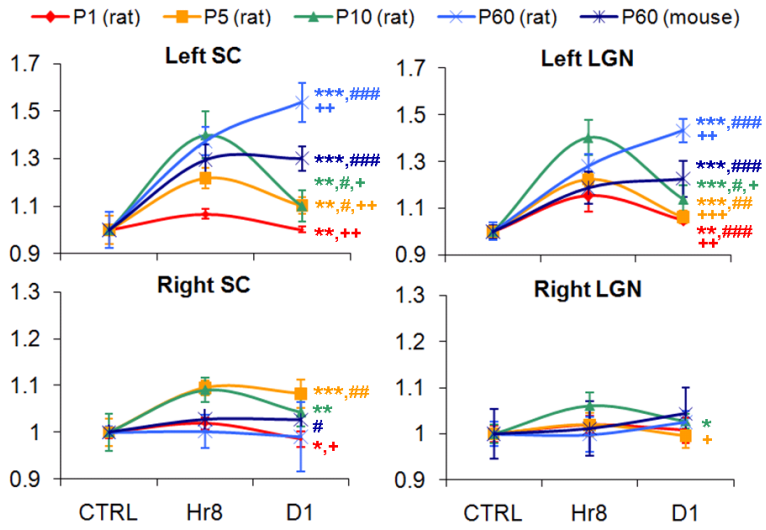

Fig. 2: Quantitative analyses of T1W signal increase in SC and LGN of each hemisphere in the same age group. (Two-tailed unpaired t-test between CTRL and Hr8, *p<0.05; **p<0.01; ***p<0.001; Two-tailed unpaired t-test between CTRL and D1, #p<0.05; ##p<0.01; ###p<0.001; Two-tailed paired t-test between Hr8 and D1, +p<0.05; ++p<0.01; +++p<0.001)

DISCUSSIONS AND CONCLUSION: The results of this study demonstrated the feasibility of in vivo, high-resolution MEMRI for assessing the retinal projection in SC and LGN of the early postnatal brains before natural eyelid opening, and suggested that the transport of Mn^{2+} is different along neonatal and adult visual pathways. Despite a lower rate of fast axonal transport in the neonatal visual pathway (8), the current results of earlier occurrence of maximal Mn-enhancement in contralateral left SC and LGN of neonatal brains than adult brains might be partially ascribed to the higher diffusion in the unmyelinated fibers of the developing optic tracts and superficial SC (9), the lower activity in the functionally immature retina, optic nerve and optic nerve synapses at SC (8), and the higher blood-brain barrier permeability in the immature rat brains (10). The SI increase in the ipsilateral right SC of neonatal rats and adult mice appeared to indicate the high sensitivity of MEMRI for detecting the ipsilateral retinocollicular projections of the newborn rat and pigmented adult mice, which are more prominent than those of the adult albino rats (11,12). Whether the little SI changes in the right LGN was attributive to partial volume effect remained to be elucidated. Future MEMRI studies are envisioned that measure the retinotopic changes along both ipsilateral and contralateral visual pathways in normal development, disease, plasticity and therapy longitudinally in the neonatal brains.

REFERENCES: 1. O'Leary DD, et al. Prog Brain Res 2005;147:43-65; **2.** **2.** Pautler RG, et al. MRM 1998;40(5):740-748; **3.**Yu X, et al. Nat Neurosci 2005;8(7):961-968; **4.** Sha O, et al. Neuroembryol Aging 2006;4:183-188; **5.** Lam K, et al. Brain Res 1982;255(3):487-491; **6.** Lowe AS, et al. NMR Biomed 2008;21(8):859-867; **7.** Thuen M, et al., JMRI 2008;28(4):855-865; **8.** Specht SC. Exp Neurol 1977;56(2):252-264; **9.** Warton SS, et al. Exp Brain Res 1985;58(3):490-502. **10.** Farrell CL., et al. Microsc Res Tech 1994;27(6):495-506; **11.** Laemle LK., et al. Brain Res 1982;255(2):317-322; **12.** Drager UC, et al., J Comp Neurol 1980;191(3):383-412.