In vivo Mapping of Retinal Projections in Rat, Gerbil and Mouse Brains using MEMRI

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INTRODUCTION: Mn^{2+} has been increasingly used as a T_1-weighted contrast agent for neuronal tract tracing (1) and functional mapping of the visual brain at lamina levels (2). Recently, increasing evidence suggested the role of retinal ganglion cells for projection not only to the visual nuclei but also to the non-visual functional nuclei (3). However, to date the sensitivity of Mn-enhanced MRI (MEMRI) for evaluating retinal projections to non-visual functional nuclei has not yet been fully explored (4). The rodents are an excellent model system for understanding the mechanisms of retinal fiber formation, degeneration and plasticity in the brain (5). In this study, we employed high-resolution 2D/3D MEMRI to examine the retinal projections to both visual and non-visual functional nuclei after intravitreal unilateral injection of $MnCl_2$ in three rodent species (rats, gerbils and mice).

MATERIALS AND METHODS: Animal Preparation: Adult Sprague-Dawley rats (226±27g, n=6), Mongolian gerbils (66±2g, n=4) and C57BL/6J mice (20±1g, n=6) were prepared. $MnCl_2$ solution (100mM) was injected intravitreally into one eye to all animals, at a volume of 2μL for rats and gerbils, and 0.5μL for mice. MEMRI was performed 1 day after Mn^{2+} administration. As more than 90% of the axons of rodent retinal ganglion cells decussate to the contralateral posterior visual components at the optic chiasm (6), the Mn^{2+} enhancement pattern in the brain components of the opposite hemisphere served as an internal control. **MRI Protocol:** All MRI measurements were acquired utilizing the 7 T Bruker scanner using a receive-only surface coil for rats and gerbils, and a mouse brain volume coil for mice. 2D spin-echo T_1-weighted (T1W) imaging was acquired in the coronal plane using RARE sequences, with TR/TE = 420/7.5ms, FOV = 32x32mm^2 for rats and gerbils, and 20x20mm^2 for mice, acquisition resolution = 125x125μm^2, slice thickness = 0.8mm, number of slices = 10, RARE factor = 4 and number of averages = 16. 3D T1W imaging was acquired using MP-RAGE sequences covering the entire visual pathway, with TI/TR/TE = 1200/9/3ms, FOV = 32x32x11mm^3 for rats and gerbils, and 25.6x25.6x9.6mm^3 for mice, acquisition resolution = 200x200x200μm^3, 1 segment and number of averages = 9. **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. The signal intensities of Mn^{2+} enhancement in the superior colliculus (SC), lateral geniculate nuclei (LGN), CA3 region of hippocampus (hipp) and posterior medial amygdala (MeP) in each hemisphere were measured in 2D T1WI for each species using ImageJ v1.42q, and were normalized to a non-visual area in the brain. Values of each brain component were compared between contralateral hemispheres in the same species using two-tailed paired t-tests. Results were considered significant when $p<0.05$.

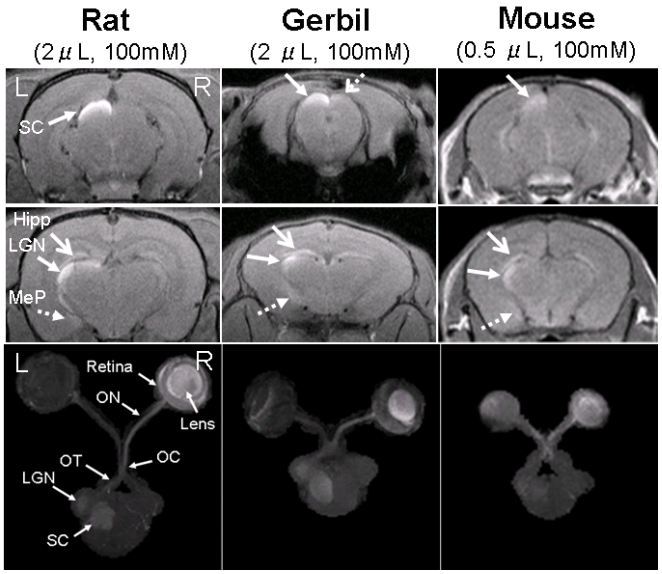

Fig. 1: (Top and middle rows) Coronal 2D T1WI of the rat (left column), gerbil (middle column) and mouse (right column) brains at the levels of the superior colliculus (SC) (top row) and lateral geniculate nucleus (LGN) (middle row) 1 day after intravitreal Mn^{2+} injection into the right eye (Hipp: hippocampus; MeP: posterior medial amygdala). (Bottom row) MIP of axial 3D T1WI of the rat (left), gerbil (middle) and mouse (right) primary visual pathways from retina to subcortex 1 day after intravitreal Mn^{2+} injection into the right eye. (ON: optic nerve; OC: optic chiasm; OT: optic tract; LGN: lateral geniculate nucleus; SC: superior colliculus)

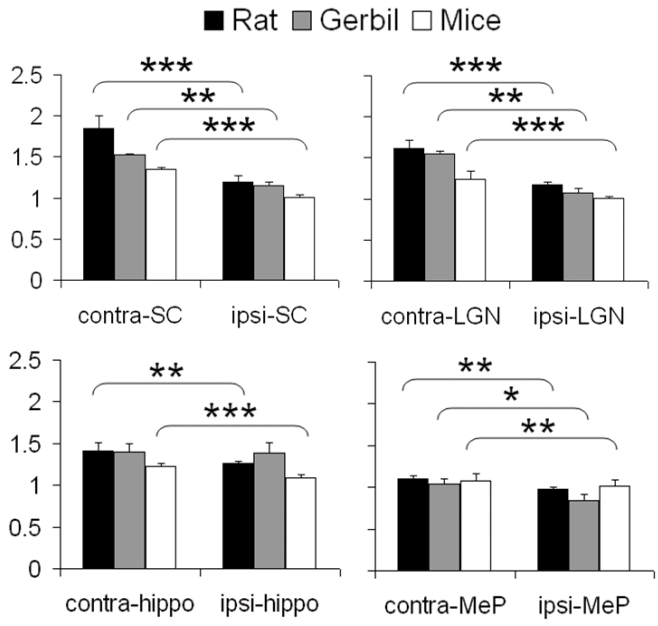

Fig. 2: Comparisons of T1W signal intensities of visual (top) and non-visual (bottom) functional nuclei between left and right hemispheres in the same species. (Two-tailed paired t-test, *p<0.05;**p<0.01;***p<0.001).

RESULTS: One day after intravitreal Mn^{2+} injection, Mn-enhancements were observed in the visual nuclei in retina, lens, optic nerve and optic chiasm of the ipsilateral brain, and in the optic tract, lateral geniculate nucleus and the superficial layers of superior colliculus of the contralateral brain in all species (Fig. 1). Mild enhancement could also be found in the ipsilateral superior colliculus (dashed open arrows) in gerbils but less in rats and mice. In addition, Mn-enhancements were observed in the non-visual nuclei especially in posterior medial amygdala (dashed arrows) and hippocampus (open solid arrows) of the contralateral brain in all species. Quantitative analyses in Fig. 2 indicated a significant signal increase in the posterior visual brain nuclei (SC and LGN), as well as in the non-visual functional nuclei in the posterior medial amygdala and hippocampus across species.

DISCUSSIONS AND CONCLUSION: The results of this study demonstrated the feasibility of MEMRI for in vivo tract tracing of the retinal projections across rodent species. In addition, we demonstrated for the first time a consistent unilateral enhancement in the posterior medial amygdala after monocular Mn injection in all rats, gerbils and mice. This appeared to be partially ascribed to a direct retinal projection from the melanopsin-expressing retinal ganglion cells (3), which are distinct from the classical photoreceptors. Whether the enhancement patterns in the hippocampus were potentially related to non-specific enhancements shown in previous olfactory tract-tracing experiments (7) remained to be elucidated. Future MEMRI studies are envisioned that measure the widespread retinal projections in normal development, disease, plasticity and therapy in longitudinal studies.

REFERENCES: 1. Pautler RG, et al., MRM 1998;40(5):740-748; **2.** Bissig D, et al., NI 2009;44(3):627-635; **3.** Hattar S, et al., J Comp Neurol 2006;497(3):326-349; **4.** Watanabe T et al., NMR Biomed 2004;17(8):554-568.; **5.** O'Leary DD, et al., Prog Brain Res 2005;147:43-65; **6.** Fleming MD, et al., Neuroscience 2006;143(3):895-904; **7.** Chuang KH, et al., MRI 2009;27(5):594-600.