# In vivo Mn-enhanced MRI for Visuotopic Mapping in Normal and Reorganized Brains

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**INTRODUCTION:** The rodents are an excellent model for understanding the mechanisms of development and plasticity in the visual system. To date, limited tools have been available for in vivo, high-resolution mapping of neuroarchitecture in the visual brains globally and longitudinally. $Mn^{2+}$ has been increasingly used as a T1W contrast agent for in vivo neuronal tract tracing (1-3) and functional brain mapping at lamina levels (4,5). In this study, we explore the capability of Mn-enhanced MRI (MEMRI) via 3 different routes of $Mn^{2+}$ administration for in vivo assessments of retinal, callosal and transsynaptic connections in normal and reorganized rat brains.

**MATERIALS AND METHODS: Animal Preparation:** Adult Sprague-Dawley rats (N=25) were divided into 4 groups. In Group 1 (n=4), a fractionated dose of $MnCl_2$ at 3μL and 50mM each was injected intravitreally into the left eye every day for a total of 3 days; In Group 2 (n=6), $Mn^{2+}$ was injected unilaterally into the left lateral geniculate nucleus (LGN) at 30nL and 100mM; In Group 3 (n=7), $Mn^{2+}$ was injected intracortically to the V1/V2 transition zone of the right visual cortex at 100nL and 100mM; In Group 4 (n=8), 4 rats underwent neonatal binocular enucleation (BE) at postnatal day 1, and 4 rats were untreated and acted as a control (CTRL). Intracortical $Mn^{2+}$ injection was performed to all rats at 3 months old with the same procedures as in Group 3. For Group 1, MEMRI was performed before, and at 1, 2 and 3 days after initial $Mn^{2+}$ intravitreal injection. For Groups 2 to 4, MEMRI was performed at 1 hour, 8 hours and 1 day after $Mn^{2+}$ administration. **MRI Protocol:** All MRI measurements were performed using a 7 T Bruker scanner. 2D RARE T1WI was acquired with TR/TE = 475/8.8ms, spatial resolution = 125x125x800 $\mu m^3$ and total scan time = 15 mins. 3D MPRAGE T1WI was also acquired at spatial resolution = 200x200x200 $\mu m^3$. **Data Analysis:** T1W signal intensities (SI) in superior colliculi (SC), lateral geniculate nuclei (LGN), primary visual cortex (V1), and V1/V2 transition zone of each hemisphere, and in the splenium of corpus callosum (CC) were measured using ImageJ v1.43u, and were normalized to the surrounding muscles. Mn enhancement was quantified by calculating the ratio between left and right visual components in Groups 1 and 2, and the rate of signal increase at Hour 8 and Day 1 compared to Hour 1 in Group 3. Values at each time point were compared to the first time point using two-tailed paired t-tests. The volumes of enhanced visual callosal projection in the left V1/V2 border contralateral to intracortical $Mn^{2+}$ injection were quantified by computing the number of pixels in the left visual cortex with T1W SI at Day 1 higher than mean + 2 standard deviations of that at Hour 1 after intracortical $Mn^{2+}$ injection, and were compared between BE and CTRL groups using two-tailed unpaired t-tests. Results were considered significant when $p<0.05$.

**RESULTS AND DISCUSSION:** In normal brains, fractionated, intravitreal $Mn^{2+}$ injection resulted in significant Mn enhancements in contralateral SC and LGN by 45-60% at 1-3 days after initial $Mn^{2+}$ injection, and in contralateral V1 (arrows) by about 10% at 2-3 days after initial $Mn^{2+}$ injection [Fig. 1 (top)]. Direct, single-dose $Mn^{2+}$ injection to LGN resulted in Mn enhancement by 8-11% in ipsilateral SC, and 13-21% in ipsilateral V1 (arrows) at Hours 8-24 [Fig. 1 (bottom)]. Intracortical, single-dose $Mn^{2+}$ injection to the visual cortex resulted in Mn enhancement by 17-25% in contralateral V1/V2 transition zone (closed arrows), 32-34% in CC (open arrows), 53-65% in ipsilateral dorsal LGN (dashed arrows) and 15-26% in ipsilateral SC at Hours 8-24 (Fig. 2). Notably, some patchy patterns were apparent near the V1/V2 border of the contralateral left hemisphere (Fig. 3), which might be indicative of the ocular dominance domains recently suggested in rodents (6). In Group 4, intracortical $Mn^{2+}$ injection to BE rats enhanced a larger projection volume by about 74% in the V1/V2 transition zone of the contralateral hemisphere compared to normal CTRL rats (Fig. 4). This suggested an adaptive change in interhemispheric connections and spatial specificity in the visual cortex upon early blindness (7).

**CONCLUSION:** The current results demonstrated the sensitivity of MEMRI for assessing the neuroarchitecture of the visual brains in vivo via 3 different routes of $Mn^{2+}$ administration without depth-limitation, and may possess great potentials for studying the basic neural components and connections in the visual system longitudinally during development, plasticity, pharmacological interventions and genetic modifications in future studies.

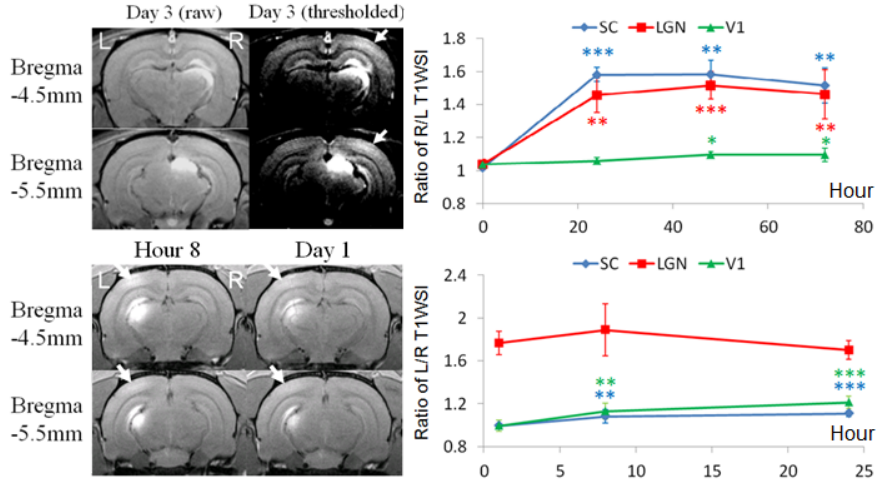


**Fig. 1: (Top row) Intravitreal, fractionated $Mn^{2+}$ injection.** (Top left) MEMRI at 3 days after initial $Mn^{2+}$ administration to the left eye. (Top right) Quantitative analyses of T1W SI between right and left SC, LGN and V1 before, and at 1, 2 and 3 days after initial $Mn^{2+}$ injection; **(Bottom row) Subcortical, single-dose $Mn^{2+}$ injection.** (Bottom left) MEMRI at 8 hours and 1 day after $Mn^{2+}$ administration to the left LGN. (Bottom right) Quantitative analyses of T1W SI between left and right SC, LGN and V1 at 1, 8 and 24 hours after $Mn^{2+}$ injection. (Two-tailed paired t-tests with 1st time point, *p<0.05; **p<0.01; ***p<0.001)

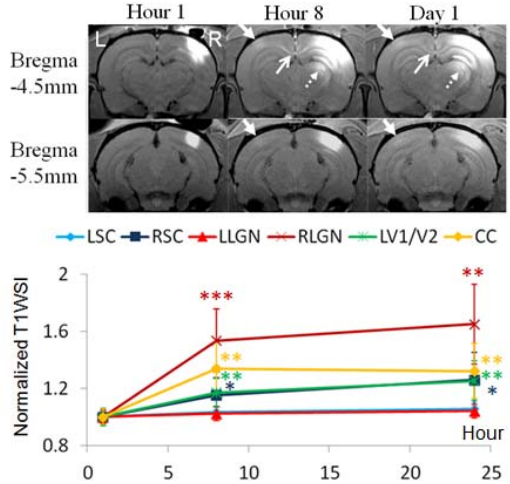


**Fig. 2: Intracortical, single-dose $Mn^{2+}$ injection.** (Top) MEMRI at 1, 8 and 24 hours after $Mn^{2+}$ administration to the right V1/V2 transition zone. (Bottom) Quantitative analyses of T1W SI of visual components normalized to Hour 1. (Two-tailed paired t-tests with Hour 1, *p<0.05; **p<0.01; ***p<0.001)

**REFERENCES: 1.** Watanabe T, et al. Mag Res Med 2001;46(3): 424-429; **2.** Tucciarone J, et al. Neuroimage 2009;44(3):923-931; **3.** Chan KC, et al. Neuroimage 2011;54(1):389-395; **4.** Bissig D, et al. Neuroimage 2009;44(3): 627-635; **5.** Yu X, et al. Nat Neurosci 2005;8(7):961-968; **6.** Liang L, et al. Abstr Soc for Neurosci 2010; 483.10; **7.** Toldi J, et al. Prog Neurobiol 1996; 48(3):191-218.

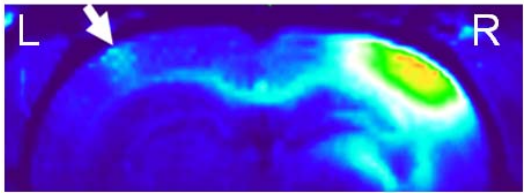


**Fig. 3:** Reconstructed 3D T1WI of dorsal brain at 8 hours after right intracortical $Mn^{2+}$ injection. Note the patchy patterns of Mn enhancement in the V1/V2 transition zone of the contralateral hemisphere (arrow) projected along the visual callosal pathway.

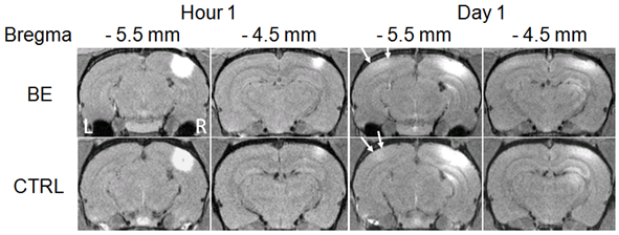


**Fig. 4:** MEMRI at 1 hour and 1 day after intracortical $Mn^{2+}$ injection to the right visual cortex of BE and CTRL rats at 3 months old. The BE group exhibited a larger area of projection near the V1/V2 border of the contralateral left hemisphere compared to CTRL group (arrows).