Retino-thalamo-cortical and callosal connections visualized with manganese-enhanced MRI and validated with tract tracing techniques

R.J. Laing^{1*}, D.J. Cross², J.F. Olavarria¹

Departments of ¹Psychology, ²Radiology, University of Washington, Seattle, WA

Introduction

We have previously demonstrated that Long Evans rats have ocular dominance columns (ODCs) in primary visual cortex (V1), which correlate with patchy callosal connections¹ (see Fig. 1). The high inter-individual variability of these columns poses a challenge for studies of ODC plasticity. Manganese (Mn^{2+}) is a paramagnetic ion that can be readily detected using MRI. Here we explore *in vivo* manganese-enhanced magnetic resonance imaging (MEMRI) as a possible strategy for longitudinal studies of plastic changes in both the retino-thalamo-cortical and callosal pathways.

Methods

Anatomical tracing:

- Retino-thalamo-cortical projections were labeled by injecting wheat-germ agglutinin conjugated to horseradish peroxidase (WGA-HRP) into one eye.
- Cortico-cortical connections were labeled by performing multiple injections of HRP into the opposite cortex.
- All tissue was sliced coronally at 60 μ m with a freezing microtome.

Manganese-Enhanced MRI:

- $MnCl_2$ was injected into one eye (1.0M) or directly into one cortical hemisphere (0.125M) either 36 or 12 hours before scans, respectively.
- Images were acquired on a 3T magnet (Philips Achieva), using a custom surface coil and a T1-weighted MPAGE image sequence over 118 coronal slices (voxel size 0.11x0.11x0.2 mm³).
- For tangential reconstructions of MRI scans, image sequences were rotated -20 degrees and re-sliced at 0.2mm thickness.

Data Analysis:

- All images were analyzed in Adobe Photoshop CS5 or ImageJ.

Transneuronal transport of Mn^{2+} visualized with MEMRI

Figure 2: MEMRI signal from an animal injected intraocularly (right eye) with $MnCl_2$ shows Mn^{2+} accumulations in the superior colliculus (SC; B,C,F,G arrow outlines), lateral geniculate nucleus (LGN; D,H arrow outlines) and cortex (A-C,E-G solid arrows). Post-scan signal intensity levels for each region were significantly higher than pre-scan levels, N = 148 slices in 3 rats ($P < 0.01$). Accumulations of Mn^{2+} in these regions corresponded with those labeled with the transneuronal tracer WGA-HRP in the same animal (I-L). Scale bar = 3mm.

Fig. 2 Manganese accumulations in SC, LGN and Cortex

Fig. 3 Post- vs. pre-scan intensity increases

Figure 3. Mean estimated percent intensity increase from pre- to post-scans for each analyzed region.

Fig. 4 Ipsilateral Contralateral

Figure 4. Re-sliced, tangential views of cortex show clear transport of Mn^{2+} to the contralateral hemisphere of V1. Black arrows indicate borders of V1 as revealed with Mn^{2+} .

MEMRI can detect abnormalities in callosal connections

Figure 5: Transcallosal MEMRI signal and HRP labeling from adult rats injected intracortically with $MnCl_2$ and HRP in one hemisphere. In normal rats, both MEMRI signal (A-C) and HRP labeling (D-F) reveal the callosal band located at the lateral border of V1 (shown with arrows only in central panel). Following monocular enucleation at birth (MEPO), both MEMRI signal (G-I) and HRP labeling (J-L) show a similar expansion of the callosal band compared to normal (Fig. 7). These results are consistent with previous reports on the effects of neonatal enucleation on the callosal pathway^{2,3}. Scale bar = 3mm.

Fig. 6 Tangential view

Figure 6: Re-sliced, MRI signal showing tangential views of the callosal band in normal and MEPO animals. Callosal bands are encircled by red lines.

Fig. 7 Width of Callosal Band

Figure 7: Comparisons of the widths of the callosal bands visualized with MEMRI and HRP between normal and MEPO rats.

Summary and Conclusions

- In addition to strong signals in the SC and LGN, we also observed transneuronal transport to visual cortex in hemispheres contralateral and ipsilateral to the eye injected with $MnCl_2$. Accumulations of Mn^{2+} in all of these locations corresponded with regions labeled with WGA-HRP in the same animal.
- Following intracortical injections of $MnCl_2$ in one hemisphere, we observed strong MRI signal in the contralateral hemisphere. The accumulation of $MnCl_2$ in both normal and MEPO rats resembled the distribution of callosal connections revealed with HRP in the same animal.
- These results suggest that both transneuronal retino-thalamo-cortical and cortico-cortical transport of Mn^{2+} provide potentially useful strategies for longitudinal studies of plastic changes in these pathways.

References and Acknowledgements

- Laing R, Turecek J, Takahata T, Olavarria JF (2015). *Cerebral Cortex* 25: 3314-3329
- Olavarria J, Malach R, Van Sluyters RC (1987). *J Comp Neurol* 260: 321-348.
- Chan KC, Cheng, JS, Fan S, Zhou IY, Yang J, Wu EX (2012). *Neuroimage* 59: 2274-2283.

Funding for this work was supported by University of Washington Department of Radiology, NIH EY16045, and Vision Training Grant: T32 EY07031.