Quantitative Assessments of Mouse Optic Nerve Projections to the Brain In Vivo

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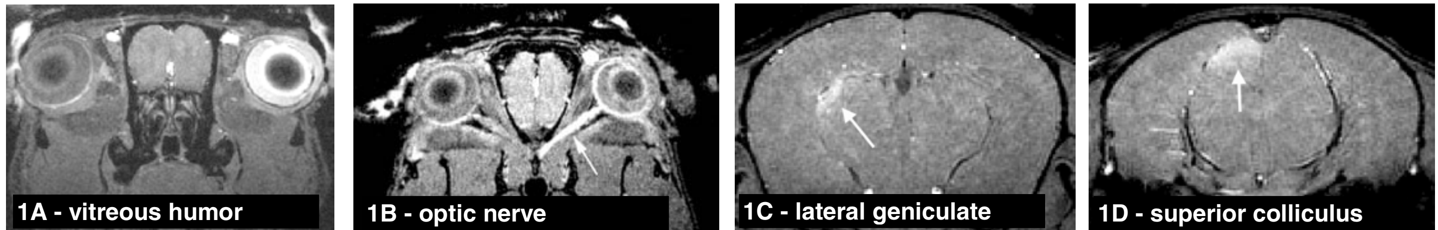
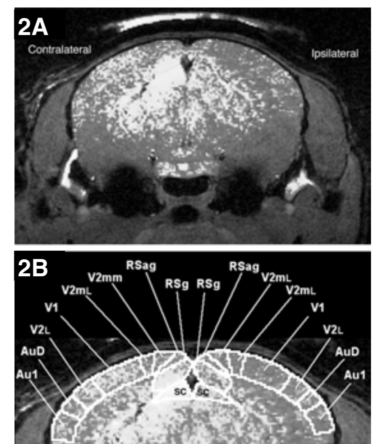
Introduction: Quantitative assessment of optic nerve axon loss or brain changes in experimental models of eye diseases such as glaucoma are typically done post-mortem in histological tissue sections. The ability to directly assess axonal integrity throughout the visual system in vivo would allow repeated evaluations within the same subject. This could permit a more accurate determination of the rate of disease progression. Recently, manganese (Mn) has been found to label neuronal tracts visualized by magnetic resonance imaging (MRI). After focal injection into the brain, Mn is transported along axons projecting both anegradly and retrogradely from the injection site. This Mn movement occurs at rates consistent with fast axonal transport. Transsynaptic Mn labeling of neurons has been previously observed in the mouse olfactory system, but not within the visual system. In the visual system, transsynaptic transfer may occur from the retinal ganglion cell to LGN relay neuron. Then to the V1 cortical neuron, and then to the V2L cortical neuron. Hence, the present study was undertaken to determine whether transynaptic labeling of the mouse visual system projection to visual cortex could be detected using Mn labeling and high field MRI.

Methods: 5 NIH-SW mice received unilateral Intracameral injections of 1 μl of 1M MnCl2 (intravitreal. est. conc. 6 μM). 5 control mice - no injection. Brain & eyes were imaged at 18 hrs at 7Tesla using T1w 3D Flash sequence. Signal intensity was measured in ROIs selected from Vitreous Humor near optic nerve, Optic Nerve adjacent to chiasm (ON), Lateral Geniculate Nucleus (LGN), Superior Colliculus (SC), Visual Cortex Area (V1) and Visual Cortex Area (V2L). Data normalized to muscle image intensity.

Results: Fig1: Mn distributed throughout the vitreous humor (excluded from the lens) to the optic nerve of injected eye, and contralateral LGN and SC.

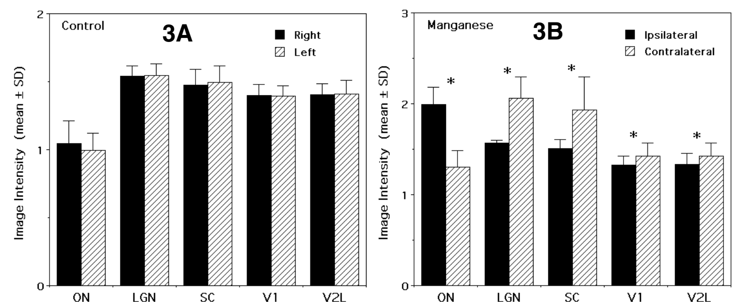


Transsynaptic delivery of Mn to visual cortex areas V1 and V2L was also observed. Fig.2A shows Mn enhancement as a narrow grayscale window, superimposed back onto the original anatomical image using Amira software. Fig. 2B shows Mn enhancement and location of murine visual cortical areas.



Comparison of control mice Image intensities in the right and left LGN of each control mouse differed by less than 2.5% (1.26% ±0.90%, mean ± SD, N = 5). In contrast, the raw mean image intensities across the different control mice differed by up to 149% (33% coefficient of variation - CV). To reduce inter subject variation, we normalized images to muscle signal. Normalization reduced CV between control mice to ~5% (Fig.3 A)

Mn signal throughout the visual system (Fig. 2B) In the control mice there was no significant difference between normalized image intensities in the ON and all analyzed brain regions (P>0.15 for all ROIs, N = 5 mice). In the experimental mice, however, image intensity was higher in the ipsilateral eye and pre-chiasmatic ON, as well as higher in the contralateral LGN, SC, and visual cortex ROIs. As shown below, statistical evaluation found these differences were significant for all ON and brain ROIs analyzed. (* indicates P<0.02 for all ROIs, paired t test, N = 5 mice).



Conclusions: 1.Mn labeling allows quantitative analysis of major visual system components in vivo and demonstrates transynaptic transfer of Mn. 2. Higher field strength allows detection of Mn in V1 and V2 visual cortex (Mn transfer across 2 synapses). 3. Normalization facilitates direct comparison of MRI image intensity scores. 4. Intracameral Mn injection provides reproducible labeling of visual system in vivo.