# In vivo Assessments of Ocular Dynamics, Axonal Transport and Microstructural Integrity in the Visual System upon Neonatal Hypoxic-ischemic Injury

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INTRODUCTION: Neonatal hypoxic-ischemic (HI) encephalopathy is a major cause of brain damage in infants and may result in periventricular white matter injury and chronic neurological dysfunctions. Infants with HI injuries frequently present cerebral visual impairments upon unilateral posterior cerebral lesions [1]. In addition, carotid artery occlusion from HI injury can cause chronic retinal and optic nerve degeneration [2]. Understanding the long-term outcomes of the remaining visual pathways after neonatal brain injury are potentially important in determining and improving the functional consequences of brain lesions before most compensatory and reparative phases have been passed. This study employed dynamic gadolinium-enhanced MRI, manganese-enhanced MRI (MEMRI) and diffusion tensor imaging (DTI) to evaluate the ocular dynamics, axonal transport and microstructural integrity in the visual system of adult rats that underwent neonatal unilateral HI injury.

MATERIALS AND METHODS: Animal Preparation: Sprague-Dawley rats (12-16 g, N=8) underwent unilateral ligation of the right common carotid artery at postnatal day 7 under isoflurane anaesthesia, followed by hypoxia in 8% oxygen and 92% nitrogen at 36-37°C for 2 hours. One year after HI insults, dynamic Gd-enhanced MRI and DTI were performed to all animals. One month after Gd-MRI, MEMRI was performed to 4 randomly selected animals. **MRI Protocol:** All MRI measurements were acquired utilizing a 7 T Bruker scanner. Under inhaled isoflurane anaesthesia (3% induction and 1.5% maintenance), animals were kept warm under circulating water at 37°C and were imaged using a receive-only surface coil. 2D T1-weighted RARE sequence was acquired at 10 to 60 minutes after intraperitoneal injection of an MRI contrast agent, Gd-DTPA (Magnevist) at 3 mmol/kg, with TR/TE = 320/10.4 ms, RARE factor = 4 and NEX = 20, FOV = 3.27 x 3.27 cm^2, voxel resolution = 64x 64 μm^2 and slice thickness = 1 mm. One month after Gd-MRI, MnCl$_2$ solution (3μL, 50mM) was injected intravitreally into both eyes of 4 randomly chosen animals. T1-weighted MEMRI was performed covering the whole visual brain at 1 day after Mn^{2+} administration. For DTI, multi-shot SE-EPI diffusion weighted images were acquired with FOV = 3.2 x 3.2 cm^2, MTX = 128 x 128, slice thickness = 1 mm, number of slices = 12, TR/TE = 3000/30ms, b = 0 and 1000 s/mm^2, number of shots = 4 and 30 diffusion directions. Fractional anisotropy (FA) maps were extracted from DTIStudio.
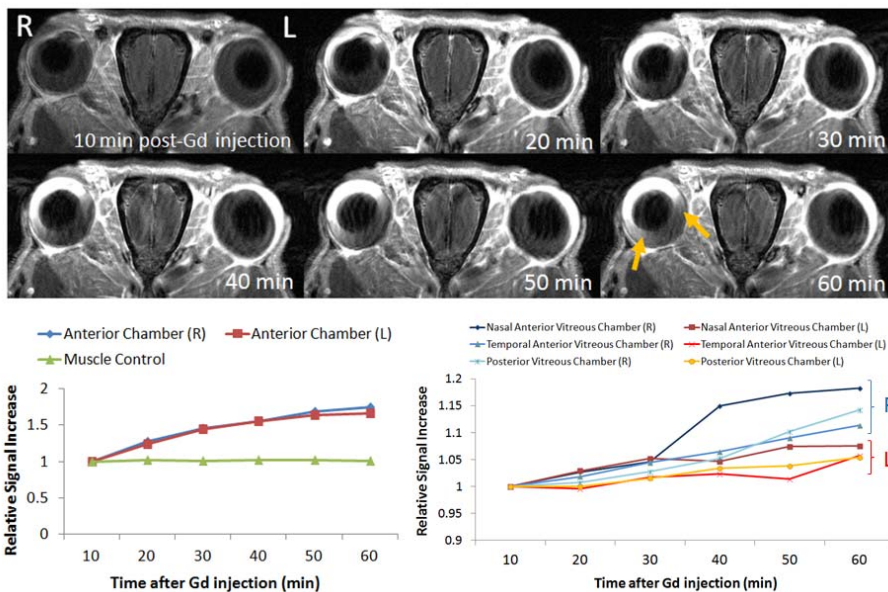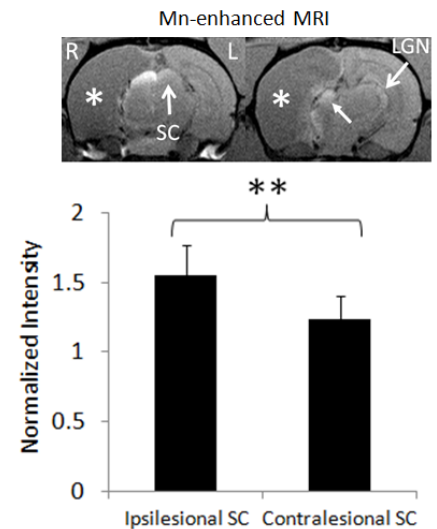

Fig. 1: Dynamic Gd-enhanced MRI of ocular transport at 10 to 60 minutes after systemic administration of Gd-DTPA. Comparable Gd enhancement was found in the anterior chamber of both eyes as expected because of the blood-aqueous barrier permeability to Gd [3]. Leakage of Gd from the anterior chamber to the vitreous was observed in the right (R) ipsilesional eye only and was not apparent in the left (L) contralesional eye.

Fig. 2: Mn-enhanced MRI showing less anterograde Mn axonal transport from the ipsilesional right eye to the contralesional left superior colliculus (SC) and lateral geniculate nucleus (LGN) compared to ipsilesional SC and LGN (paired t-test, **p<0.01). Lesion is indicated by an asterisk.

RESULTS AND DISCUSSION: Dynamic Gd-enhanced MRI showed leakage of the Gd contrast from the anterior chamber into the vitreous humor in the ipsilesional right eye but not the contralesional left eye after systemic administration of Gd (Figure 1), likely in relation to the compromise of permeability at the blood-aqueous barrier and/or aqueous-vitreous interface [4]. MEMRI showed less anterograde axonal transport of Mn^{2+} ions along the visual pathway projected from the ipsilesional eye compared to contralesional eye upon bilateral Mn^{2+} intravitreal injection (Figure 2). In addition, the right lateral geniculate nucleus ipsilateral to the injured hemisphere appeared to be compressed and displaced by the lesion. The MEMRI findings may be supported by DTI findings which showed more disorganization of the contralesional left optic tract projected from the ipsilesional eye compared to the ipsilesional right optic tract (Figure 3). The results of this study may provide important evidence for understanding the chronic pathophysiological changes in the visual system after neonatal unilateral HI injury and for improving strategies for vision preservation.

REFERENCES: [1] Lanzi G, et al. Neuropediatrics 1998;29(3):145-150; [2] Stevens WD, et al. Stroke 2002;33(4):1107-1112; [3] Kolodny NH, et al. Invest Ophthalmol Vis Sci 1996;37(8):1602-1607. [4] Chan KC, et al. Exp Eye Res. 2008 Oct;87(4):334-41.

Fig. 3: DTI showed a lower FA in the contralesional optic tract (OT) projected from the ipsilesional eye compared to the ipsilesional OT (paired t-test, **p<0.01).