Manganese Enhanced MRI of Optic Nerve Damage and Regeneration in Rats

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Introduction: Conventional optic tract tracing relies on sectioning of the brain, which exclude repetitive measurements of the same animal. Manganese is a calcium analog, which is taken up and transported along the nerve. Mn^{2+} is paramagnetic and acts as a MRI contrast agent by shortening the T1 relaxation time (1). In this study manganese was injected into normal and damaged optic nerves to study Mn^{2+} enhancement during regeneration. Axons in the central nervous system do not generally regenerate, but it has been shown that introducing a nerve graft into the proximity of the nerve can stimulate regeneration to some degree (2).

Materials and Methods: *Animals and Experimental Protocol:* Inbred female Fisher rats (100–115 g at start) were used. The experiment consisted of four experimental groups: one pilot group of 10 rats for evaluation of dose-response (the dose-response group), one group of 5 rats with no optic nerve damage (the control group), one group of 10 rats which received an optic nerve crush without nerve graft (the optic nerve crush group), and one group of 10 rats that had their optic nerve crushed and a nerve graft implanted into the vitreous of the left eye (the nerve graft group). In the dose-response group the rats received intravitreous injections of respectively 0.1, 1, 2, 5, 7.5, 10, 25, 50, 75 and 100 mM Mn^{2+}, and MRI was performed 24 hours after injection. In all other groups, a concentration of 100 mM Mn^{2+} was injected. In the control group, MRI was performed 24, 48, 96 and 168 hours after Mn^{2+} injection. In the optic nerve crush group the optic nerve was crushed and the rats were investigated twice, both two days after the nerve crush and 20 days later. At both investigations MRI was performed 24 hours after Mn^{2+} injection. In the optical nerve graft group the nerve was crushed and a nerve graft was implanted. MRI was performed 20 days after optic nerve crush (24 hours after Mn^{2+} injection). Before surgical procedures, manganese injection and MRI, the animals were anaesthetized with a 1:1:2 mixture of Hypnorm/Dormicum/Sterile water subcutaneously (0.047 ml/g) after 2-3 minutes initial breathing in mixture of O_2 and 4 % isofluran.
Mn^{2+} Injections: Based on the pilot study, a concentration of 100 mM Mn^{2+} was used. Aqueous solution of $MnCl_2$ was injected into the vitreous body of the left eye using a purpose-built injection device consisting of a plastic syringe connected via polyethylen tubing to a glass micropipette with a tip diameter of ~10 microns.
Optic nerve lesions and nerve grafts: The optic nerve was accessed intracranially with the aid of a microscope after a left-sided frontal craniotomy and crushed for 10 seconds 2 mm behind the eye. One Fisher rat was sacrificed and the sciatic nerve was used as a graft for 10 animals. The nerve was grafted immediately after removal and each graft had the length of 1 mm. The graft was introduced into the vitreous body through a perforation in the sclera, 1.5 mm dorsal to the optic disc.
MRI: MRI was performed at 2.35 T using a Bruker Biospec Avance DBX-100 (Bruker AG, Ettlingen, Germany) with a 72 mm volume coil for transmission and an actively decoupled quadrature rat head surface coil for receive-only. Water-cooled BGA-12 (200 mT/m) gradients were used. The animals were anaesthetized and placed headfirst prone in the magnet using a dedicated animal bed. Warm air was passed through the magnet to maintain a body temperature of 37°C. A sagittal multislice gradient-echo image with 10 slices was performed for planning of a 3D volume: FOV=5×5 cm, matrix=128×128, TR=150 ms and TE=6 ms. The 3D data set was obtained using a T1-weigthed 3D gradient-echo sequence (FLASH) with TR=15 ms and TE=4.2 ms, and a flip angle of 25 degrees. A field of view of 5×5 cm in both rostal-caudal and left-to-right directions, and 2 cm in the dorsal-ventral direction were used. With an acquisition matrix of 256×256×128, the voxel resolution was 195×195×156 μm^3. 8 averages were used and the total acquisition time was 65.5 minutes.

Data analysis: The 3D-images were analyzed using ParaVision 3.0 (Bruker AG). In a 2D reconstruction of the 3D volume, regions of interest were drawn manually and mean and standard deviation of the ROIs were found. The contrast to noise ratio was calculated from $CNR=0.655 \cdot C/SD_{air}$; $C=S_{Mn}-S_0$, where S_{Mn} and S_0 are the signal intensities in the Mn^{2+} enhanced and opposite nerves, respectively. SD_{air} is the mean value of the standard deviation in two regions in air (3).

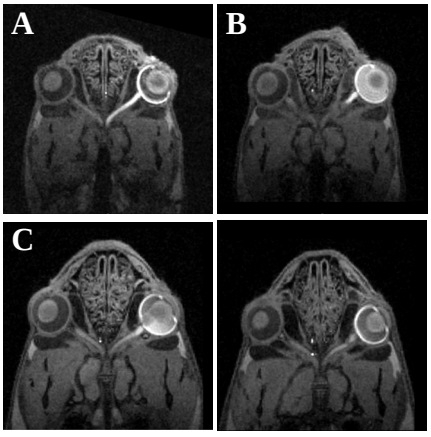

Figure 1. *Manganese enhanced optic nerves. (A) Control rat and (B) rat with optic nerve crush. MRI was performed 2 days after the crush. (C) Rat with optic nerve crush and nerve graft and (D) rat with optic nerve crush only. MRI was taken 20 days after optic nerve crush was performed.*

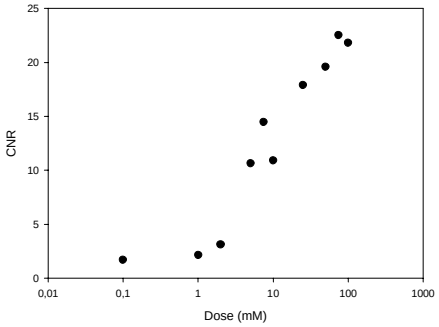

Figure 2. *CNR in the enhanced optic nerve as a function of Mn^{2+} dose 24 hours after Mn^{2+} injection. A visible enhancement of the Mn^{2+} injected nerve was seen at 5 mM and higher, and there was a log-linear relationship between the dose of Mn^{2+} and the increased signal intensity in the enhanced nerve. Each point reefers to the contrast to noise ratio in the optic nerve in one rat.*

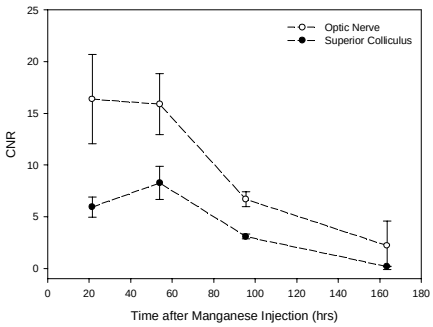

Figure 3. *Dynamics of manganese contrast in optic nerve and superior colliculus after Mn^{2+} injection. In the optic nerve the maximal intensity was found 24 hours after injection, and the intensity decreased and reached the baseline after one week. In the SC the maximal intensity was found 48 hours after injection. MRI was performed on 2-4 rats at each point in time, and the graph shows the mean and standard deviation of CNR.*

Results and discussion: MRI performed 24 hours after Mn^{2+} injection in the control group showed an enhanced signal along the optic nerve from the retina to the superior colliculus (SC). In the damaged nerve there was no manganese enhancement beyond the lesion site, supporting the hypothesis that manganese is transported along the axons (figure 1 a-b). A reduction in the dose of Mn^{2+} by a factor of 10 gives a visible enhancement of the optic nerve, and there was a log-linear relationship between the Mn^{2+} dose injected and the contrast to noise ratio (figure 2). After one week the manganese had visually disappeared from the optic nerve (figure 3). 20 days after the optic nerve crush was performed, the Mn^{2+} uptake is reduced, also in the retina. This implies that the optic nerve crush kills some of the retinal ganglion cells. Analysis of the MR images taken 20 days postoperative shows higher uptake of Mn^{2+} in the optic nerves stimulated with nerve grafts compared with animals without implants (t-test; p=0.047) (figure 1 c-d). MR images will be compared with conventional fluorescent tracer microscopy of dissected nerves to verify our results.

Conclusion: This study demonstrates that Mn^{2+} can be used to image the optic nerve from the retina to the superior colliculus, and that the manganese stops at a mechanically induced crush site. This suggests that Mn^{2+} can be used to study regeneration after CNS damage.

Reference: (1) Pautler RG et al. Magn. Reson. Med. 40: 740-748 (1998), (2) Berry M et al. J Neurocyt. 28: 721-741 (1999), (3) Firbank MJ et al. Phys. Med. Biol. 44: N261-N264 (1999)