Detection of axon regeneration by manganese enhanced magnetic resonance imaging in the adult rat optic nerve after intravitreal peripheral nerve grafting

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Introduction: Axons in the mammalian peripheral nervous system regenerate after injury, while those in the central nervous system (CNS) do not. A method that has shown to be one of the most effective to stimulate regeneration in the optic nerve (ON) in adult rats is the implantation of a peripheral nerve graft (PNG) into the vitreous body of the eye, after which retinal ganglion cell (RGC) axons do regenerate in the putative inhibitory environment of the transected ON in vivo[1,2]. Manganese (Mn^{2+}) is paramagnetic and acts as an MRI contrast agent mainly by shortening the T1 relaxation time. In addition, Mn^{2+} is a calcium analogue that is taken up by Ca^{2+}-channels and transported anterogradely along the ON axonal microtubules. Axonal Mn^{2+}-transport is halted after transection of the ON[3]. Most studies of ON regeneration so far have used traditional tract tracing methods that required that the animals are sacrificed before tissue sectioning. Here, we show that MEMRI can be used for in vivo, longitudinal studies of regeneration in the adult rat ON.

Materials and Methods: *Animals:* Adult inbred female Fischer rats (n=10) were used in the study. Optic nerve crush (ONC) was performed with a pair of microforceps intraorbitally 2 mm caudal to the lamina cribrosa according to the method described by Berry et al.[4] A segment of sciatic nerve was implanted intravitreally in 9 animals in the left eye through a perforation in the sclera 1 mm dorsal to the optic disc immediately after ONC. Immediately following the surgical procedures the animals received an intravitreal Mn^{2+}-injection of 150 nmol $MnCl_2$ (3 µl x 0.05 M) through the ora serrata. The Mn^{2+}-injection was repeated 20 days later. For surgical procedures, Mn^{2+}-injections and MRI, rats were anaesthetized with a 1:1:2 mixture of Hypnorm/Dormicum/sterile water subcutaneously (0.025 ml/g). The Norwegian ethical committee guidelines for animal research were followed and all experiments were approved by the responsible governmental authority.

MEMRI was performed 1 d and 21 d after ONC at 7 T using a Bruker Biospec Avance 7 (Bruker Biospin 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 (400 mT/m) gradients were used. For scanning, animals lay prone in a dedicated animal bed with circulating hot water (37 °C) within the magnet. A 3D data set was obtained using a T1-weighted 3D low flip angle gradient-echo sequence (FLASH) with TR=12.5 ms and TE=3.7 ms, and a flip angle of 20º. The acquisition matrix was 192×192×112 giving a resolution of 208x208x205 μm^3. Eight averages were used and the total acquisition time was 35.84 min. A set of two low resolution 3D data sets were obtained for non-uniform corrections using the surface and volume coil for receive, respectively.

Figure 1

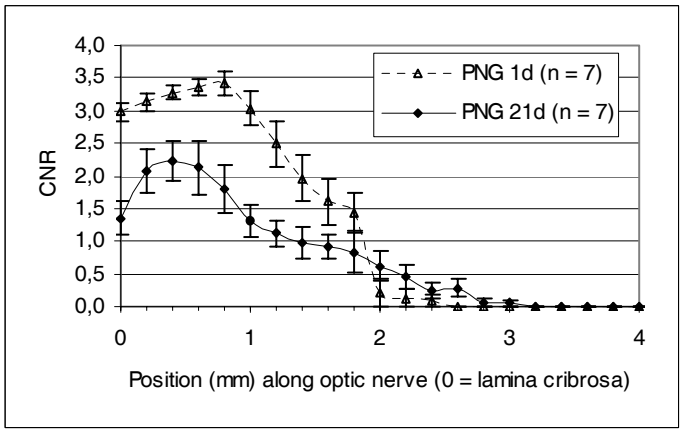

Figure 1: CNR of ON with crush at 2 mm caudal to the lamina cribrosa and PNG implanted in the vitreous body 1 day (PNG 1d) and 21 days (PNG 21d) after the optic nerve crush. Errorbars represent ± SE of mean.

Figure 2

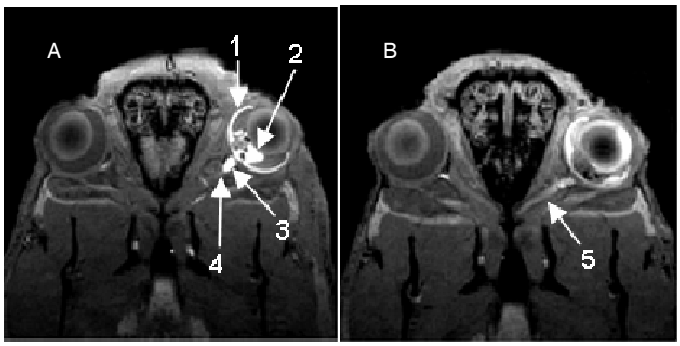

Figure 2: MEMRI of retina (1), PNG (2) and ON (3) 1d (A) and 21d (B) after ONC. No Mn^{2+} enhancement was seen distal to the crush site (4) 1d after the surgery while an enhancement distal to the crush site was seen 21 days later (5).

Data analysis: The non-uniform features of the 3D dataset caused by the surface coil were corrected using the corresponding low resolution datasets. The ON was segmented and the contrast to noise (CNR) profiles calculated by re-sampling the 3D image-volume in 2D planes perpendicular to the Mn^{2+} enhanced ON in 0.2 mm steps, 4 mm along the segmented ON measured from the lamina cribrosa. A mean signal of the nerve in each 2D plane was calculated. The signal from the non-enhanced ON was subtracted and the CNR calculated. To compare the CNR 1 d and 21 d after ONC and PNG implantation a paired t-test was used. A 5 % significance level was established using SPSS 14.0.

Results and Discussion:
The segmentation and CNR calculations resulted in the CNR profiles given in Figure 1. The mean CNR difference in the interval 0.0 mm – 1.8 mm between 1 d and 21 d after surgery was 1.197 ($p < 0.001$) with a 95 % confidence interval (CI) = (0.960, 1.435). Distal to the crush site, in the interval 2.0 mm – 3.0 mm, the CNR 21 d after surgery was > CNR 1 d after surgery. The mean difference was 0.202 ($p = 0.006$) with a 95 % CI = (0.065, 0.359). This result indicated that Mn^{2+} was transported through the crush site and further anterogradely along the optic nerve 21 days after the nerve crush. Our results correspond well with the histological findings reported by Berry et al.[4] in the same animal model.

These findings demonstrate that MEMRI can be used in vivo longitudinal studies for detecting regeneration of RGC axons in the adult rat ON, and promises to be an effective method for studying axon regeneration in the mammalian CNS.

References:

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