Manganese-enhanced MRI of Axon Regeneration by Peptide Nanofiber Scaffold Induction

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INTRODUCTION: Manganese (Mn^{2+}) has been being used for *in vivo* axonal tracing of the rodent visual pathways in MRI because of its paramagnetic property and its ability to enter intracellular space as a calcium analogue [1]. Previous studies showed the feasibility for Mn^{2+} to detect optic nerve lesions and regeneration by nerve graft implantation to the site of an acute injury via T1-weighted MRI [2,3]. While axons in the injured central nervous system (CNS) of adult mammals have been shown to regenerate upon peripheral nerve transplantation, this technique requires sectioning of the sciatic nerve from the legs, which often results in leg disabilities in experimental models [4]. In this study, Mn^{2+}-enhanced MRI (MEMRI) was performed to demonstrate the reconnection of brain tissues in a chronic injury without grafting. A self-designed, self-assembly peptide nanofiber scaffold (SAPNS) was applied to a severed optic tract which had been left untreated for 3 months. The SAPNS created a tissue-bridging structure, connected the two sides of lesion and allowed movement of cells into the scaffold in nanoscale [5].

METHODS:

Experimental Procedures: The optic tract was transected at the brachium of the left superior colliculus (BSC) of adult Syrian hamsters (130-175g, n=8) 3 months before SAPNS treatment [5]. During treatment, small cuts were made across the old transaction, and 30μL of 1% SAPNS solution was to be injected into the knife wounds. MEMRI was performed 1 week before and 1.5 months after the treatment. 4 hamsters without any transections were scanned as controls.

Intravitreal Mn^{2+} injections: Approximately 24 hours prior to MR imaging, the hamsters were anaesthetized with intraperitoneal injection of a mixture of ketamine (70mg/kg) and xylazine (7mg/kg) and a glass micropipette was inserted into the intravitreal space of the right eye at the temporal retinal margin. 0.2M of $MnCl_2$, dissolved in milliQ water was injected to a volume of 2μL. The micropipette was held inside the eyeball for about 20 seconds before pulling it out to prevent bubble formation.

3D MR Imaging: All MRI measurements were acquired utilizing a 7T Bruker scanner. Under inhaled isoflurane anaesthesia (3% induction and 1.5% maintenance), animals were kept warm at ~35°C on the heating pad and were laid prone in the same position. Images were acquired using an isotropic T1-weighted 3D FLASH sequence with TR/TE = 21.7/3.1ms, 30° flip angle, acquisition matrix = 168x168x128 and voxel resolution 226x226x226μm^3. 4 averages were used and the total acquisition time was 23.5 minutes.

Data Analysis: Maximum intensity projection (MIP) was performed on a segmented volume covering the superior colliculi (SC) and their brachii in order to investigate into the Mn^{2+} enhancement at the dome-shaped SC globally (Figure 2). Regions of interests (ROIs) were drawn manually in the SC and the area proximal to the BSC on both sides, and the mean and standard deviation of the ROIs were found using ImageJ (Wayne Rasband, NIH, USA). The ROIs were copied for each sample and each value was compared using both ratio between mean of opposite components and contrast-to-noise ratio (CNR) [$(SI_{right} - SI_{left})$/Mean of SD_{air}], where SI represents signal intensity and SD represents standard deviation [3].

RESULTS AND DISCUSSION:

1. After BSC cut for 3 months, the SI in the left SC dropped by 3 times ($p<.01$) while that in the area proximal to the knife wound was raised ($p<.05$ axially). As Mn^{2+} travels mainly via fast axonal transport [1], the data suggested an axonal loss in the transected lesion, causing the inability of organelles, including Mn^{2+}-carrying microtubules, to cross the lesion and reach the SC. Mn^{2+} had likely stayed in the end of the intact axonal pathway and accumulated along the cut edges giving out bright signals (Figure 1b).

2. 1.5 months after SAPNS treatment, the SI at the treated SC increased significantly by about 10% (Figures 3 & 4). This indicated a potential early restoration of neural connection across the site of a chronic injury. It has been shown the SAPNS not only permitted significant axonal growth through the site of the treated lesion, partially restoring the optic tract, but also resulted in the return of functional vision in brachium transected experimental adult animals [5]. The allowance for the passage of organelles and Mn^{2+} ions from the optic tract via the reinnervation to the SC might have given rise to the enhancement in the treated SC and the moderate decrease in accumulation of Mn^{2+} proximal to the transection site ($p = 0.13$). Mn^{2+} might have also been transported trans-synaptically across new functional synapses [1,5]. Results on the same set of data using contrast-to-noise ratios correlated with the above statements (not shown). Limitations in this study included Mn^{2+} neurotoxicity, while the interactions between SAPNS and Mn^{2+} will be further investigated.

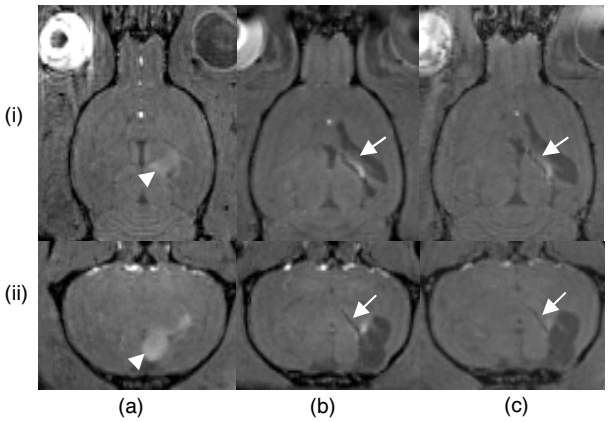

Figure 1: Typical T1W images of the hamster brain in both coronal (i) and axial (ii) views, showing clear Mn^{2+} contrasts in the left superior colliculus (SC, arrowheads) and lateral geniculate nucleus of the brain with no transection (a). Accumulation of Mn^{2+} ions can also be observed proximal to the knife cut before (b) and after (c) SAPNS treatment (arrows).

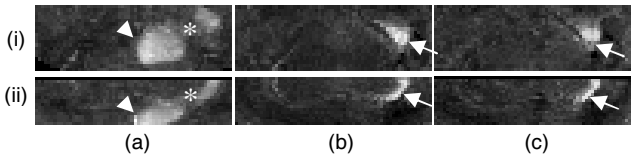

Figure 2: MIP images of the SC with the same labels as in Figure 1. A mild enhancement was found at the left SC after SAPNS treatment (c) compared to that before treatment (b). '*' indicates the area proximal to BSC.

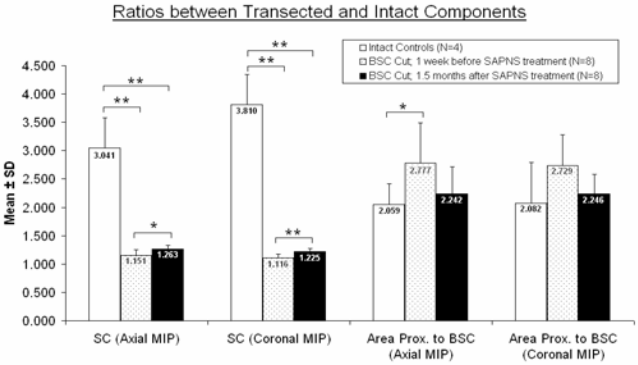

Figure 3: Comparison between ratios of transected to intact sides of the superior colliculus (SC) and the area proximal to the brachium of SC (BSC) in both axial and coronal MIP views. (two-tail paired t-test, * $p<.05$; ** $p<.01$.)

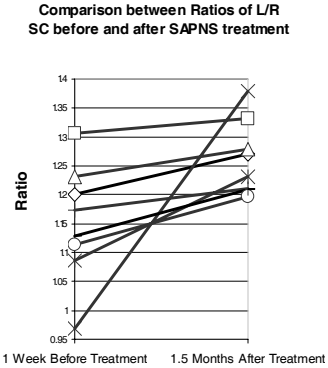

Figure 4: Ratios between transected and intact SC signals before and after SAPNS treatment. Wilcoxon signed rank test gives a p value of <0.01. (2-tail, n=8)

CONCLUSION: Manganese can serve as an MRI tracer for neuroregeneration across surgical knife cuts, while MEMRI can help monitor the reinnervation of SC using SAPNS non-invasively, longitudinally and quantitatively.

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