TRANSCRANIAL APPLICATION OF MANGANESE CHLORIDE ENABLES NEURONAL TRACT TRACING USING MEMRI

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Target audience: MRI researchers interested in neuronal tract tracing using Manganese Enhanced Magnetic Resonance Imaging (MEMRI).

Purpose: There has been growing interest in using MEMRI for neuronal tract tracing in rodents[1-2]. For this MEMRI application, manganese solutions are usually directly injected into specific brain regions[1-2]. Recently it was reported that manganese ions can diffuse through intact rat scull[3]. Here the local manganese concentrations in the brain tissue after transcranial manganese application were quantified and the effectiveness of tracing from the area was determined.

Methods: Eleven adult male Sprague-Dawley rats (body weight ~ 200 g) were used in this study. The animals were anesthetized with 5% isoflurane, and then switched to 1-2% isoflurane for maintenance. The animals were placed in a stereotaxic apparatus, a single midline incision with a sterile scalpel was made through the skin of the skull, and the skull bone was exposed by scraping away the periosteum. The following sterile saline solutions were placed directly on the skull bone above S_1 area (left/right: +3, rostral/caudal: -1.4, from Bregma): 500mM $MnCl_2$, 250mM $MnCl_2$ and pure saline as a control. The solutions were pipetted throughout the course of 2h. Images were acquired on an 11.7 T/31 cm horizontal magnet (Agilent, Oxford, UK) interfaced to a Bruker Avance III console (Bruker BioSpin, Billerica, MA) immediately after manganese application as well as 24h later. Contrast enhancement by manganese was detected using a T_1-weighted spin echo pulse sequence (TE=7.6 ms, TR=500 ms, Nav=8, 100 μm in-plane resolution, thirty 1-mm thick axial slices). The T_1 relaxation times were measured using a saturation recovery spin-echo sequence (TE=7.6ms, TRs=0.4; 0.97; 1.77; 3.124 and 10s, 200 μm in-plane resolution). All the data processing was performed using ImageJ (http://rsb.info.nih.gov/ij/) and MIPAV (http://mipav.cit.nih.gov/) image processing software developed at NIH. T_1 relaxation maps for brain slices were calculated using the MRI Analysis Calculator plugin. Background tissue T_1 values and standard deviations for every slice were obtained by placing a region of interest (ROI) over an area of the cortex opposite from the one of manganese administration. T_1 relaxation maps were thresholded using the value of the background T_1 minus two standard deviations in order to obtain ROIs in which manganese had significantly shortened T_1 of the cortex tissue. The average T_1 relaxation times as well as total number of voxels in those ROIs were used to determine the local concentration and total amount of manganese in each slice, using the equation: $1/(T_1)_{obs}=1/(T_1)_{backgr} + r_1*c$. The previously reported value of 4.7 s^{-1} mM^{-1} was used for cortex T_1 relaxivity[4] . The total amount of manganese was integrated through the relevant slices. T_1 relaxation maps were also thresholded using the value of the minimum T_1 in the ROI plus two standard deviations of the T_1 of the background tissue that did not receive any manganese in order to calculate the highest local concentration of manganese, using the same equation as above. Thalamus ROIs were manually drawn using rat brain atlas.

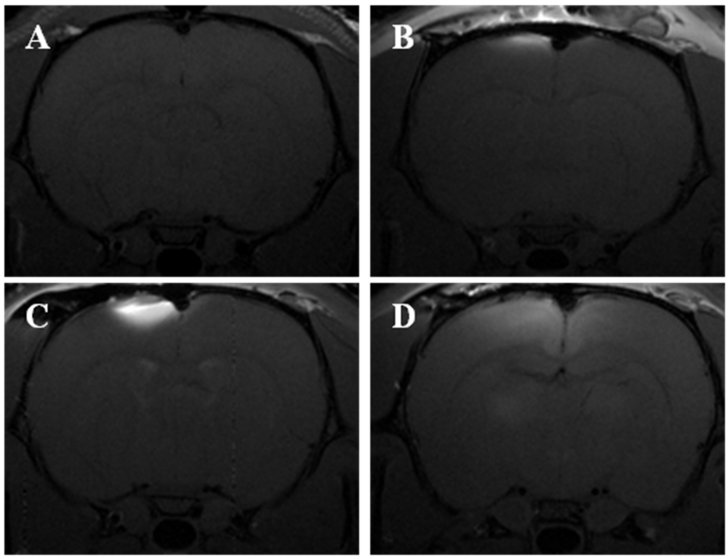

Figure 1: T_1-weighted images of rats receiving vehicle solution (A), 250 mM $MnCl_2$ (B), 500 mM $MnCl_2$ (C) immediately after solution application, as well as of 500 mM $MnCl_2$ 24h after administration (D).

Results: The effectiveness of the manganese diffusion through intact rat scull is demonstrated by the images in Fig. 1B and C, where hyperintense regions represent cortex areas with significant manganese concentrations. Using T_1 values from Fig. 2, it was determined that the average cortex manganese concentrations were 45±9 and 74±2 μM, for 250 and 500 mM applied solutions, respectively. The highest local concentrations of manganese were determined from areas with minimal T_1 values to be 330±100 and 560±70 μM. Fig. 1D demonstrated enhancement in the corticothalamic pathway 24h after manganese administration. Average T_1 values in the thalamus decreased by 8 and 30%, for 250 and 500 mM applied manganese solutions, respectively.

Discussion: This study shows that it is possible for manganese ions to diffuse through the intact rat scull upon application on the top of the scull bone. The local manganese tissue concentrations are proportional to the concentrations of topically applied manganese solutions. Manganese ions introduced into S_1 cortex in this manner cause enhancement in the corticothalamic neuronal pathway, analogously to manganese ions directly injected into S_1 area[5].

Conclusion: Transcranially applied manganese yields brain tissue enhancement patterns similar to those obtained when manganese is directly injected into desired brain area. This represents a new and much less invasive manganese delivery method for neuronal tract tracing using MEMRI.

References: **1**. R.G.Pautler et al., Magn Reson Med, 40:740-748, 1998; **2**. R.G. Pautler et al., NMR Biomed,17: 595-601, 2004, **3.** T.L.Roth et al., Nature, in press, **4**.K.H.Chuang et al., Magn Reson Med, 61:1528-1532, 2009, **5.** T.B.Leergaard et al., NeuroImage 20 (3): 1591–1600, 2003.

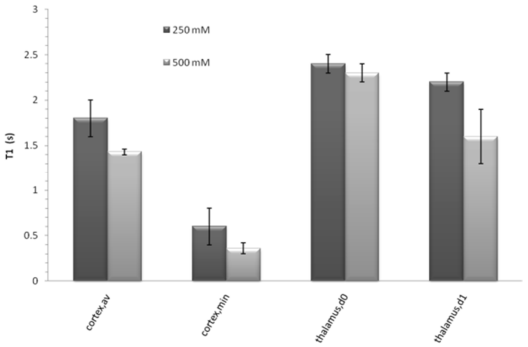

Figure 2: Calculated T_1 values in the cortex and thalamus immediately after manganese application (d0), as well as in thalamus 24h after administration (d1).