A Surface Diffusion Method for Cortical Mn Administration in MEMRI

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INTRODUCTION: Manganese (Mn) enhanced MRI (MEMRI) has been proved to be effective for studying brain activity, tracing neuronal pathways and layer-specific cortical microstructures [1]. The invasiveness of Mn administration methods, however, may cause neuropathological changes, contaminating *in vivo* MEMRI investigation of neural functions. Efforts have to be made to balance among toxicity of Mn, invasiveness and signal enhancement efficiency, including optimizing the injection volume, concentration, infusion rate, etc. [2, 3]. Recently, others have demonstrated effective transmeningeal transport of neuronal transmitters [4-6]. Here, we demonstrated that Mn can also be uptaken by neocortex through spontaneous diffusion for effectively tracing layer specific corticortical and thalamocortical connections.

MATERIALS AND METHODS: Animal Preparation: Adult Sprague-Dawley rats (300~320g, n = 24) were divided into 3 groups. In the injection group (n=10), rats were injected with 0.1 uL of $MnCl_2$ solution (500mM) into the right visual cortex at 6.5mm posterior, 5.5mm lateral from bregma with 1.0mm depth from cortical surface. In the single spot diffusion group 2 (n=9), the cortical surface was exposed by drilling a whole with 1.0mm in diameter also at 6.5mm posterior and 5.5mm lateral to bregma. Care was taken to avoid injuring the cortex. Then a piece of 1mm × 1mm sterile absorbable medical sponge was immersed in 2uL $MnCl_2$ solution (with concentration of 250mM, 500mM and 1M, respectively) and applied over the exposed cortex. In the tangential application group (n=5), rats' skulls were drilled tangentially open with a width of 0.5mm from 6.5mm posterior and 0.5mm anterior, and 3.5-5.5mm lateral to bregma. Then 5 uL of $MnCl_2$ solution (with concentration of 500mM or 1M) was applied similarly through diffusion facilitated by sponge. MEMRI was performed before injection and at 1 day after injection. **MRI Protocols:** All MRI scans were performed using a 7T Bruker MRI scanner with a surface coil. During the scan, rats were anaesthetized with isoflurane (3% for induction and 1.5% for maintenance) with respiration monitored and were kept warm using circulating water at 37°C. Modified driven equilibrium Fourier transform (MDEFT) images were acquired with TR = 4000ms, Echo TR/TE = 12/4ms, FOV = 32x32mm^2, MTX = 256x256, slice thickness = 0.5mm, number of slices = 28 and number of averages = 6. RARE T_1 images were acquired using the same pixel dimensions, with TR/TE = 400/7.5ms, slice thickness = 0.9mm, number of slices = 9, RARE factor = 4 and number of averages = 28. **Data Analysis:** Signal intensity (SI) in left V1/V2 TZ, right visual cortex, posterior corpus callosum (CC) and right lateral geniculate nuclei (LGN) was measured using ImageJ and normalized by the background noise to minimize difference in animals. 3D spatial Mn distribution maps of the brain were generated using AMIRA. Mann-Whitney test was performed with $p < 0.05$ considered to be statistically significant.

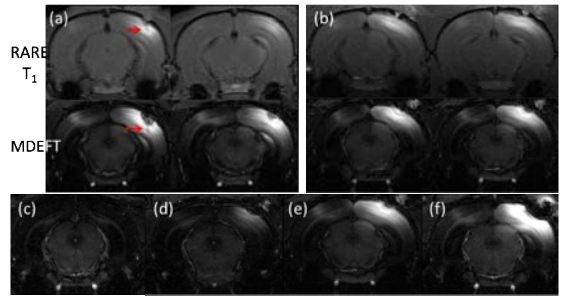

Fig. 1 (a) RARE T_1 and MDEFT images of the injection group (Mn concentration: 500mM; injury marked by red arrows). (b) RARE T_1 and MDEFT images of the diffusion group (Mn concentration: 500mM). MDEFT images of the diffusion group (Mn concentration: 250mM (d), 500mM (e) and 1M (f)) acquired at post-surgery day 1 compared with the normal group (c).

RESULTS: At post-injection day 1, enhancements were observed in left V1/V2 TZ, CC and right LGN as shown in MDEFT images. Fig.1a and Fig.1b show the comparison between the injection method and the single spot diffusion method. Note the intact brain surface as revealed by RARE T_1WIs using the diffusion method. Fig.1c-f illustrates the SI enhancements in left V1/V2 TZ after applying $MnCl_2$ solutions of different concentrations using the single spot diffusion method. Significant layer specific enhancements appeared with the concentration of 500mM and 1M. Fig. 2a-b shows ROI definition for signal quantification. As shown in Fig. 2c, there was no statistical significant difference between the single spot injection and diffusion methods in SI of left V1/V2 TZ, CC and LGN (normalized by the SI of right visual cortex). Fig. 2d demonstrates signal the ROIs were significantly enhanced after single spot manganese application through surface diffusion. Fig. 3a shows the exposed tangential intact cortical surface for applying $MnCl_2$ solution by diffusion. Fig. 3b demonstrates brain connectivity at post-surgery day 1 within multiple pathways, including visual (V1/V2 transition zone), somatosensory and cortico-thalamic-spinal motor pathway.

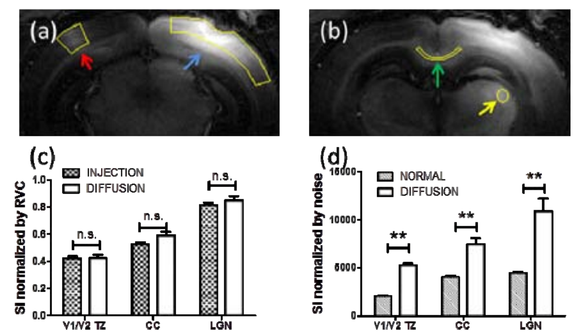

Fig. 2 ROIs are highlighted in (a) and (b): V1/V2 transition zone (the red arrow), visual cortex (the blue arrow), CC (the green arrow) and LGN (the yellow arrow). (c) Comparison between the injection and diffusion groups in MDEFT signal intensity (normalized by right visual cortex). (d) Comparison between the diffusion and normal groups in MDEFT SI (normalized by background noise). Mann-Whitney test was performed with *$p<0.05$, **$p<0.01$.

DISCUSSIONS AND CONCLUSION: This study demonstrates that the surface diffusion approach can be an alternative to the intracortical injection method previously employed in MEMRI. The dosage of Mn solution was optimized by balancing between maximizing signal enhancement and minimizing toxicity, while the volume was chosen to avoid injury as well as to lengthen duration of enhancement. At post-surgery day 3, SI enhancements could still be noted in ROIs listed above. Compared with the latter, our approach appears to be less invasive (Fig.1a). No significant difference was observed in SI enhancements and in animals' post-surgical behaviors between diffusion and injection of the same concentration. Additionally our approach allows large dosage application for tracing long-distance connections, e.g. corticospinal pathway, which was previously difficult by intracortical injection.

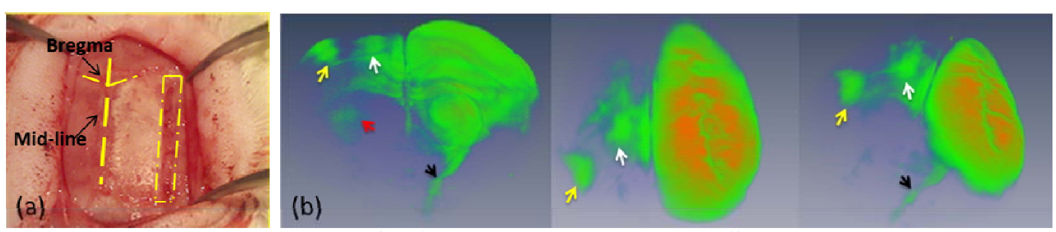

Fig. 3 (a) Tangentially exposed cortical surface for manganese administration via diffusion (circled by the rectanglar) . (b) 3D spatial distribution map of signal intensities at post-surgery day 1. Several structures were enhanced due to inter-or intrahemispheric manganese transfer including V1/V2 transition zone (yellow arrows), primary somatosensory cortex trunk region (white arrows), thalamus (the red arrow) and motor pathway (black arrows).

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