ACTIVITY-MODULATED INTERHEMISPHERIC MANGANESE TRANSFER UPON INTRACORTICAL INJECTION

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INTRODUCTION: Manganese enhanced MRI (MEMRI) has been increasingly applied for measuring neuronal activity and tract tracing[1]. Tucciarone et al. demonstrated that MEMRI could detect layer specific interhemispheric somatosensory connections[2]. Interhemispheric visual connections are also highly organized with layer specificity as revealed by tremendous fruitful electrophysiological and histological studies[3,4]. However, such studies are often laborious and not appropriate for *in vivo* and longitudinal investigations. Unlike the other cortical areas, the neuronal responses of visual cortex are strictly different from its subcortical inputs[5]. Such uniqueness enables experimenters to differentiate cortical and subcortical changes upon manipulation and ask fundamental questions about cortical development and plasticity. This study aims to test whether inherhemispheric visual connections could be characterized by high resolution 3D MEMRI with efficiency and specificity, and whether Mn^{2+} transfer could be modulated by visual activity changes.

METHODS: **Animal Preparation:** 500mM, 100nl Mn^{2+} (pH=7.4) was injected slowly to primary/secondary visual cortex transition zone (V1/V2 TZ) in right hemisphere, at 6.5mm posterior and 5.5mm lateral to bregma, in 12 normal male Sprague Dawley rats and 10 monocularly enucleated (ME) rats (350-400g). The normal rats were imaged at 24 hours before, 8 hours and 24 hours after injection. The ME rats were imaged at 24 hours after injection. ME was performed at 7 days prior to Mn^{2+} injection by removing the left eye[6]. Buprenorphine (0.1mg/kg) was given subcutaneously for pain relieving for 3 days after surgery. **MRI Protocols:** All the MRI experiments were performed on a 7T Bruker MRI scanner with a receive-only quadrature surface coil. During imaging, rats were anesthetized with isoflurane (3% for induction and 1.5% for maintenance) with respiration monitoring and kept warm at 37°C. Modified Driven-Equilibrium Fourier Transform (MDEFT) images were acquired for visualizing Mn^{2+} enhancement with TR=4000ms, echo TR/TE=12/4ms, TI=1100ms, FOV=32×32mm^2, matrix=256×256, slice thickness=0.5mm, number of slices=28, number of segments=4 and averages=8. **Data Analysis:** MDEFT images from normal animals were normalized by background noise and reconstructed three-dimensionally using AMIRA for better visualization of Mn^{2+} transfer. For quantification, manually drawn ROIs were placed in the left V1/V2 TZ, posterior corpus callosum (CC), right lateral geniculate nuclei (LGN), and right visual cortex by referencing to a rat brain atlas[7]. Signal intensities (SI) of these ROIs were measured in ImageJ. SI of the left V1/V2 TZ, posterior CC and right LGN were normalized by that of the right visual cortex for statistical comparisons between the normal group and the ME group.

RESULTS: As shown in Fig 1A, V1/V2 border (green lines) and left V1/V2 TZ (encircled by the yellow box) were identified in MDEFT images acquired before Mn^{2+} injection by referencing to literatures[3,7]. After right cortical injection, Mn^{2+} enhancement in the left hemisphere was observed as a narrow bi-laminar stripe (green arrows), clearly corresponding to the V1/V2 TZ, which was evident at 8 hours after injection and further increased at 24 hours. Color-coded multi-slice and 3D MDEFT images in Fig. 1B demonstrated prominent Mn^{2+} enhancement in CC at 8 hours, which formed a thick band (yellow arrows) that bended rostrally at the midline and connected with the left and right V1/V2 TZ at each end. Layer-specific enhancement was analyzed by plotting the SI through the cortical depth (Fig. 1C) and tangentially along the cortical surface (Fig. 1D) at 8 hours and 24 hours after injection, respectively. At both time points, peak SI were detected in lower layer II/III and layer V. At 24 hours after injection, slightly SI increase was observed at V1/V2 border than that at 8hrs, while the lateral and medial areas showed more evident enhancement increase. Fig. 2A illustrates the ROI definitions in CC, LGN and right visual cortex for statistical analysis. As shown in Fig. 2B, no statistical difference was observed in CC and LGN enhancement between the normal group and the ME group, while the left V1/V2 TZ was significantly less enhanced in the latter group.

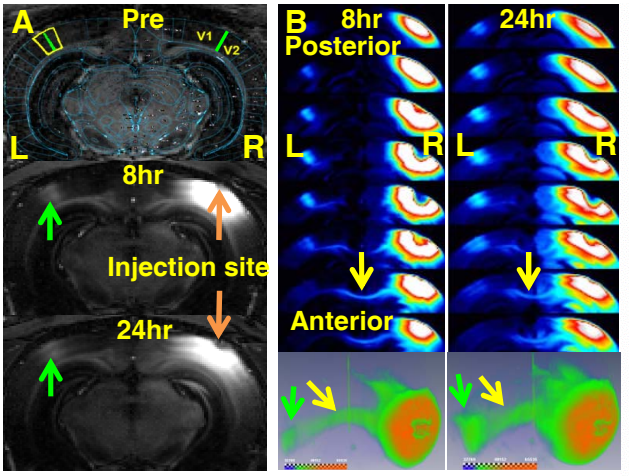

Fig. 1 Mn^{2+} transfer to the left hemisphere upon injection into the right V1/V2 TZ in normal rats. A) 2D MDEFT images acquired before (pre) and after Mn^{2+} injection. Mn^{2+} enhancement in the left hemisphere concentrated in V1/V2 TZ (yellow box and green arrows); B) Multi-slice and 3D MDEFT images showing corpus callosum enhancement (yellow arrows), and spread of Mn^{2+} enhancement in the left V1/V2 TZ (green arrows). C) Layer-specific Mn^{2+} enhancement through cortical depth. D) Tangential distribution of Mn^{2+} enhancement within left V1/V2 TZ.

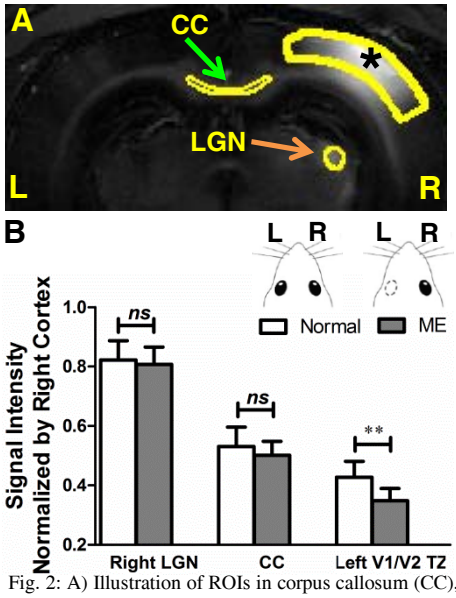

Fig. 2: A) Illustration of ROIs in corpus callosum (CC), lateral geniculate nucleus (LGN) and right visual cortex (star). B) Comparison of Mn^{2+} enhancement in right LGN, CC and left V1/V2 TZ in normal (n=12) and ME rats (n=9). $^{**}P<0.01$, two-tailed Mann-Whitney tests. ***ns***, not significant. Data were shown as mean ± SD.

DISCUSSIONS AND CONCLUSION: In this study, after locally injected into the right visual cortex, Mn^{2+} was transferred to the left hemisphere and concentrated within a narrow stripe with a bi-laminar pattern. This was in consistence with classic histological observations that callosal axons sending from the right hemisphere collectively terminated in layers II/III and V in the left V1/V2 TZ[3]. Upon left eye enucleation, making the right cortex largely deprived of visual stimulation[6], the Mn^{2+} transfer dropped by 18.5%. Note that Mn^{2+} enhancement in CC and LGN through direct anterograde and retrograde axonal transfer showed no significant decrease. These results echoed with the findings by Bearer EL et al and supported that electrical activity may not be necessary for uptake and monosynaptic transport of Mn^{2+}, but was required for its multiple synaptic transmission[8]. In summary, this study for the first time demonstrated that MEMRI is capable of tracing layer-specific transcallosal connectivity of visual cortex and is sensitive to activity modulation. Combined with newly available Mn^{2+} administration methods, including infusion with engineered minipumps, MEMRI will enable both long-term and short-term *in vivo* studies with negligible toxicity, and expediate the research into cortical development and plasticity.

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