In vivo Tracing of Cortical Laminar Structure in the Rodent Olfactory System using Manganese-Enhanced MRI (MEMRI)

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Introduction: It has been shown that manganese-enhanced MRI (MEMRI) could be used to perform anterograde neuronal tract tracing in a number of neural systems, including olfactory pathway [1,2]. Recently, laminar structure in the somatosensory cortex could also be imaged by MEMRI [3,4]. The purpose of this study was to determine if MEMRI could distinguish laminar specificity in the olfactory system and trace the layer-specific inputs from peripheral to cortex. $MnCl_2$ were infused to nostrils of rats, and they were imaged at several time intervals after infusion. After 48 h, manganese enhancement enabled detection of the layer structure of the anterior olfactory neuron (AON), piriform cortex (PC) and orbitofrontal cortex (OFC). Careful timing of the MRI allowed specific laminar inputs to be distinguished along this pathway.

Methods: To examine Mn^{2+} enhancement at high spatial resolution with good sensitivity, a Magnetization Prepared Rapid Gradient Echo (MP-RAGE) sequence was used. Sequence parameters such as optimal inversion delay time for best tissue contrast were determined from the T1 values obtained from the Look-Locker T1 mapping [2] in a pilot study. Detail imaging setup is similar to previously described [4]. Briefly, all images were acquired with an 11.7T/31cm horizontal bore magnet (Magnex, Abingdon, UK), interfaced to an AVANCE III console (Bruker, Billerica, MA). The 3D 100-µm isotropic MP-RAGE imaging was performed with the following parameters: FOV= 2.56x2.56x1.28 cm, matrix 256x256x128, TR= 4000 ms, Echo TR/TE = 15/5 ms, TI= 1000 ms, number of segments= 4, Averages= 4. The total acquisition time was 136 min. Five male SD rats were used in this study. $MnCl_2$ (500mM, 20 µl) were infused to bilateral nostrils of rats while they were anesthetized by isoflurane. In addition to a pre-infusion scan, post-infusion images of the same animal were acquired immediately, 6h, 12h, 24h, and 48h after $MnCl_2$ infusion.

Results: Compared to the pre-infusion image, Mn^{2+} enhancement was detected in the olfactory related regions at different time points. Figure 1a shows a coronal slice from the 3D MP-RAGE image of a rat brain at 48 h after $MnCl_2$ application. Intensity differences that correlate with different layers in AON (green arrows) and OFC (red arrows) are clearly visible. Figure 1b shows the images at different time points. Mn^{2+} reached AON at 12 h. After 24 h, Mn^{2+} enhancement could be seen in OFC. Figure 2a shows a horizontal slice across PC at 12, 24, & 48 h after infusion. Mn^{2+} reached anterior PC (red arrow) at 12 h and posterior PC (green arrow) at 24 h. It seems that Mn^{2+} arrived the superficial layer earlier than the deep layer. This result is consistent with histological evidence [5] that the superficial cell layer of PC receives afferents from the olfactory bulb. Laminar structures in anterior and posterior PC were evident 48h after infusion as shown in Figure 2b & 2c, respectively. The borders of PC could be defined by the intensity as well as the layer structure. The orange arrow indicates the border between PC and insular cortex, and the blue arrow indicates the border between PC and olfactory tubercle.

Conclusions: Using 3D MP-RAGE sequence, MEMRI could be used to visualize laminar structures in olfactory pathway *in vivo*. In addition, layer-specific input to olfactory cortices could also be observed by scanning at several time intervals after Mn^{2+} application to the nostrils of rats.

References: [1] Pautler et al, MRM 40:740-748 (1998). [2] Chuang et al, MRM 55:604-611 (2006). [3] Silva et al, J Neurosci Methods, 167: 246-257 (2008). [4] Tucciarone et al, NeuroImage, in press (2008). [5] Price, J. Comp. Neur. 150: 87-108 (1973).

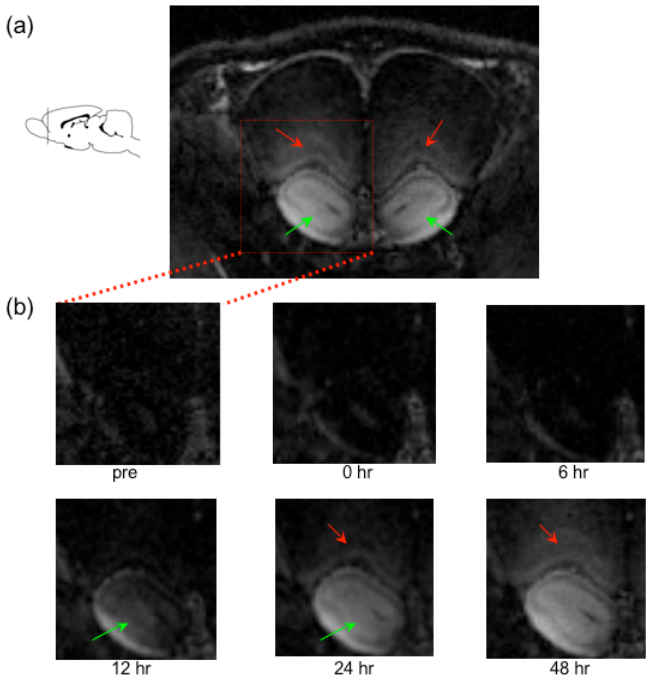

Fig 1. (a) MEMRI showed laminar structures in AON (red arrows) & OFC (green arrows) after 48 h of Mn^{2+} infusion. (b) Images acquired at different time points could be used to trace Mn^{2+} arrival time at different regions.

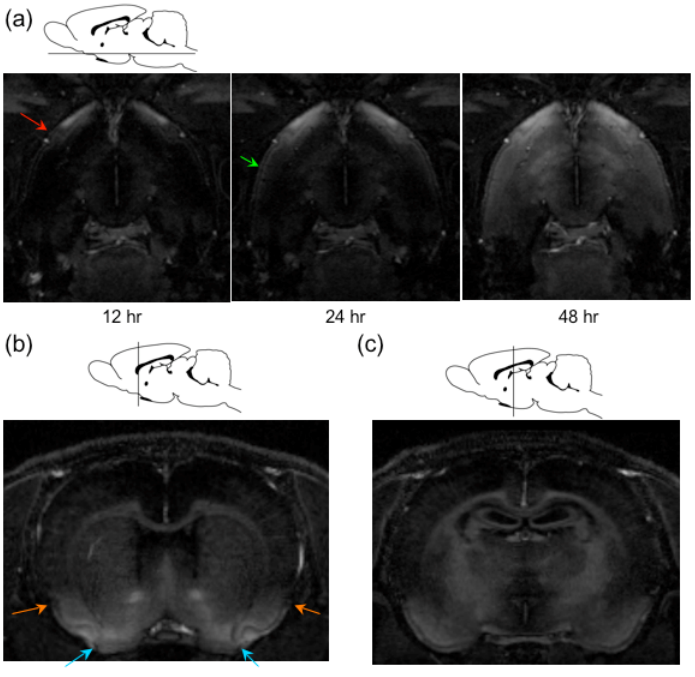

Fig 2. (a) The anterior PC was enhanced by Mn^{2+} earlier than posterior PC. In addition, Mn^{2+} arrived at the superficial layer of PC earlier than the deep cell layer. After 48 hr of Mn^{2+} infusion, layers in the anterior PC (b) and posterior PC (c) were clearly evident.