# Manganese-Enhanced MRI Tracing of Laminar Specific Functional Thalamo-Cortical Connections in Rat

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**Introduction:** Manganese-Enhanced MRI (MEMRI) has been shown to be an excellent method to trace functional neuronal connections *in vivo* [1]. With the goal of determining the anatomical localization of functional hemodynamics, we have been using $MnCl_2$ as a marker of cortical architecture in rat somatosensory cortex [2]. Although systemic infusion of manganese chloride (MnCl2) to the brain shows specific cortical lamina architecture [2], the exact mechanisms of laminar contrast are not known. Electrophysiology and microscopy studies have demonstrated that cortical lamina IV is the main area for cortical input from thalamic nuclei, with more than 80% of such afferents projecting to lamina IV [3]. The thalamo-cortico pathway is amenable to be traced with $MnCl_2$ *in vivo*. Therefore, the present study was designed to test if $MnCl_2$ as a tracer of the direct pathway between the thalamus and the rat somatosensory cortex could detect specific laminar input.

**Materials and Methods:** Five male Sprague-Dawley rats (160 – 170 g) were orally intubated and mechanically ventilated. Their heads were carefully secured to a stereotaxic frame (Kopf Instruments), the scalp was removed and the skull exposed. A 1 mm drill bit was used to make two small burr holes 3.8 mm posterior and 2.6 mm lateral to bregma. An ultra thin glass pipette containing 50 mM $MnCl_2$ was lowered 6 mm into the brain, and 100 nL of this MnCl2 solution was delivered at about 3 nL/sec. After injection, the pipette was left in the brain for an additional 5 minutes. During the entire procedure, the rat's vibrissae contra-lateral to the injection site were stimulated with 8 Hz air puff, using a home-built air puffer machine, since t has been previously shown that $Mn^{2+}$ accumulates in active regions of the brain [4]. The glass pipette was slowly removed and the burr holes were sealed with bone wax. The animals were allowed to recover from anesthesia and returned to their cages for a period of 15 hours. The animals were re-intubated, anesthetized with 2 % isoflurane and set into an MR-compatible cradle for MRI.

*MEMRI:* All MRI experiments were performed in a horizontal 11.7T/31 cm magnet (Magnex Scientific, Ltd., Abbingdon, UK), interfaced to a Bruker Avance MRI console (Bruker-Biospin, Billerica, MA). A standard multi-slice $T_1$-weighted spin-echo sequence (TR/TE=300/7 ms, in-plane resolution: 100x100µm$^2$, slice thickness: 1000 µm) was used to evaluate contrast and identify regions enhanced by $Mn^{2+}$. High-resolution 3D-MEMRI, spin-echo $T_1$-weighted (TR/TE=300/8 ms, 150 µm isotropic resolution was used to follow $Mn^{2+}$ in the somatosensory cortex.

**Results and Discussion:** Figure 1 shows consecutive coronal $T_1$-weighted images of two different rats acquired approximately 16 hours after $MnCl_2$ injection from two different rats. Rat #1 (Fig. 1, top row) was injected both in the right and left hemispheres, while rat #5 (Fig.1, bottom row) was injected into the right hemisphere only. The injection sites are marked by arrows. An overall increase in intensity could be observed in somatosensory cortex compared to other brain regions. The inset images show examples of such enhancement in cortical intensity. To verify the specificity of enhancement to cortical laminae, maximum intensity projection (MIP) images were calculated from the $T_1$-weighted spin-echo images, while taking care to avoid contamination by blood vessels. Two MIPs are shown in Figure 2. They show a distinctly bright stripe located 600-700 µm below the pial surface, along the expected anatomical location for lamina IV.

The presence of somatosensory stimulation during and after injection of MnCl2 seems to be an essential component of the successful results demonstrated here. Such presentation of a relevant stimulus during $MnCl_2$ administration probably increased the rate of uptake of Mn2+ ions by excited neurons in the thalamus, making such uptake, and thus the neuronal tracking of the thalamo-cortical projections more specific to the functional pathway. As we hypothesized, the enhancement in the MEMRI signal was found restrictedly where the thalamo-cortical projection terminates, in lamina IV.

Through the use of MEMRI we were able to detect the initial stages of cortical input from the thalamus. A similar strategy was recently used to track functional connections due to specific odors in the mouse olfactory system [5]. Our results suggest that the MEMRI method is capable of detecting local functional pathways, and alternations in neuronal connectivity during plasticity.

**References:** [1] Pautler R, Silva AC, Koretsky AP. Magn. Reson. Med. 1998, 40:740-48;Saleem KS et al, Neuron 2002, 34:685-700; Van der linden A et al Neuroscience. 2002, 112:467-74. [2] Silva AC et al, Proc. ISMRM Eleventh Meeting. 2003, p. 496; [3] Herkenham M, Science, 1980, 207:532-535; [4] Lin YJ and Koretsky AP, Magn. Reson. Med. 1997, 38:378-388; [5] Pautler R and Koretsky AP Neuroimage 2002,16:441-8.

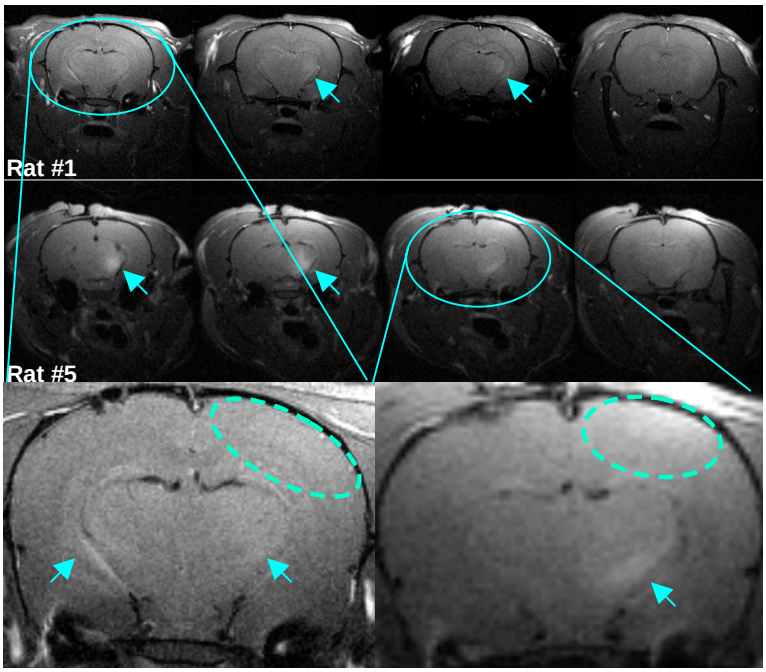


**Fig 1:** $T_1$-weighted coronal images of the rat 16 hours following the injection of $MnCl_2$ into thalamus (arrows). The cortex shows brighter (inset), due to the tracing of manganese in the thalamo-cortical projections.

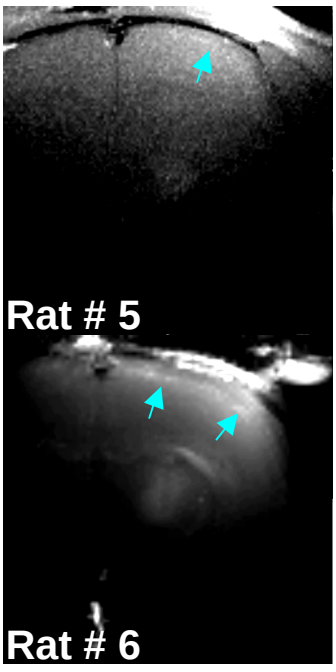


**Fig 2:** Maximum-intensity projections of $T_1$-weighted coronal images of the rat show a distinct bright stripe (arrows) located 600-700 µm bellow the pial surface, at the expected anatomical location for lamina IV.