# Functional Mapping of Rat Visual Cortex Following Light Stimulation Using Manganese-enhanced MRI

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## Introduction

Visual system is an important and well-known example of brain function. The well-defined relationship between animal visual cortical activity and light stimulation makes this system a unique model to study neuronal function and plasticity. Response to light stimulation has been investigated by non-invasive methods, such as positron emission tomography (PET) [1] and blood oxygen level dependent functional MRI (BOLD fMRI) [2]. However, application of the above functional mapping methods to rat cortex remains challenging because stimulation should be given during scanning. Appropriate anesthesia and stable hemodynamic conditions must be maintained throughout the whole course of experiment. In the study, we sought to establish an alternative working protocol of applying manganese-enhanced MRI (MEMRI) to map the visual cortex following light stimulation. In the results, we have mapped rat visual cortex using MEMRI and have shown a clear relationship between manganese enhanced cortical regions and light-evoked activity. It will be potentially useful to study plasticity in surgically or genetically manipulated rat brains.

## Materials and Methods

Adult Wistar rats were initially anesthetized with 2% isoflurane mixed $O_2$ gas. $MnCl_2$ was given, 25 mM in 10 μl, directly in to brain by stereotaxic instrument and microinjection systems. The injection rate was 0.18 μl/min and total injection time was 56 min. The rats in the experimental group (N=5) received light stimulation for three days afterward without anesthesia. Rats were scanned three days after $MnCl_2$ administration. The rats in the control group (N=5) underwent the same procedure as the experimental group without light stimulation. During the MR scanning, the rats were anesthetized with 2% isoflurane mixed with $O_2$, maintained with 1.5% isoflurane. Rat body temperature was maintained at 37°C using warm water circulation.

The experiment was performed on a 1.5T MRI system (Sonata, Siemens MAGNETOM, Germany). A surface coil was used for RF reception. Two imaging sequences were performed to acquire T1W images and R1 mapping. Multi-slice spoiled gradient echo (GE) sequences were performed to obtain T1W images with TR/TE = 17/7.57 ms; flip angle = 70°; in-plane resolution = 195μm x 390μm; slice thickness = 1.5 mm. To improve detection sensitivity over the full extent of $Mn^{2+}$ concentrations, image data for R1 mapping were acquired [3]. To obtain R1 mapping, multi-slice turbo spin echo (TSE) sequences were performed with half in-plane resolution; slice thickness = 1.5 mm; TE = 15 ms. The TSE sequence was performed to acquire 8 sets of images corresponding to 8 different TRs, ranging from 490 to 5000 ms, to sample along the recovery of longitudinal magnetization.

Given the current field strength and scan time, cortical enhancement could not be visualized clearly in each individual rat; the enhancement could only be detected by averaging the rat images of the same group of rats. The post-processing of the image data entailed stereotactic coregistration, pixel intensity normalization and inter-subjects averaging. To highlight the activity-related functional enhancement following light stimulation, the averaged $Mn^{2+}$-enhanced T1WIs in the control group were subtracted from that in the experimental group after coregistration. In addition, voxel-based t-value mapping was implemented to provide statistical difference between two groups.

## Results and Discussions

In the experimental group, cortical enhancement was localized in the visual areas (Fig. 1a), whereas in the control group the enhancement was uniform throughout the cortex showing no specific localization (Fig. 1b). To localize the activity-related areas, R1 mapping was made. In the experimental group, the R1 mapping showed gradients of R1 with their peaks located at the visual areas (Fig 1c). In the control group, the R1 mapping showed no specific localization of the gradients (Fig. 1d). Subtracted images (Fig. 1e) and voxel-based statistical t-value mapping (Fig. 1f) between experimental and control groups showed additional enhancement concentrated in the visual cortex. In the subtracted enhanced areas unrelated to light stimulation such as hippocampus, hypothalamus and amygdala were suppressed. Only the visual areas which were activated during light stimulation remained enhanced. The result was consisted with the brain atlas (Fig. 1g) [4].

In this study a clear relationship between $Mn^{2+}$-enhanced cortical regions and visual light-sense-evoked activity was demonstrated using the MEMRI method. Activity in cortex was recorded by $Mn^{2+}$ injection of followed by light stimulation outside the MRI room for three days and scanned. $Mn^{2+}$ accumulation remained even after activity stopped demonstrating the memory capability of MEMRI. The fact that $Mn^{2+}$ enters neurons during stimulation and does not leave rapidly, underlines its unique capability of mapping functioning neurons. $Mn^{2+}$ can be injected into animals and taken up by the active neurons in response to stimulation closer to their normal behavior, which can be mapped and observed by MRI afterwards [5]. Traditional MEMRI requires disruption of normal physiology by breaking brain-blood barrier (BBB), which may in turn affects normal flow. We used direct brain injection to avoid the requirement of BBB disruption.

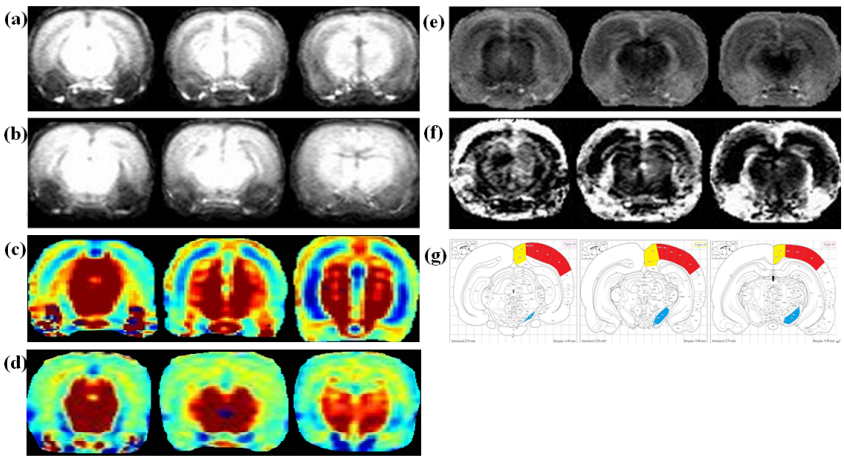


Fig. 1 Three consecutive slices of the averaged $Mn^{2+}$-enhanced T1WIs in the experimental (a) and control (b) groups. R1 maps in the experimental (c) and control (d) groups. Image subtraction (e) and voxel-based t-value mapping (f) between experimental and control groups. Red areas were the correlated visual cortex in the rat brain atlas (g).

## Conclusions

We have mapped the activated visual cortex using the MEMRI method on a clinical MR system, showing a clear relationship between $Mn^{2+}$ enhancement and visual light-sense-evoked activity. Our results indicate that the MEMRI method is potentially useful to study plasticity in surgically- or genetically- manipulated rat brains.

## References

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