

Mn-Enhanced MRI of Neural Activity in the Mouse Midbrain

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Synopsis

Manganese (Mn)-Enhanced MRI (MEMRI) has been proposed as a method to visualize neuronal activity in animals, taking advantage of the permeability of voltage-gated calcium channels to Mn^{2+} . We tested the sensitivity of MEMRI to detect activation of the mouse auditory system, imaging mice with 3D T1-weighted spin echo MRI at defined time points after intra-peritoneal (IP) injection of $MnCl_2$ and exposure to repetitive auditory stimulation. This easily-implemented protocol resulted in significant (10-15%) MEMRI enhancement in the auditory midbrain, compared to deafened control mice, showing the efficacy of MEMRI for detecting auditory-evoked neural activity in the mouse brain.

Introduction

MEMRI has been proposed as an approach to image neural function in living animals [1]. The permeability of voltage-gated calcium channels to Mn^{2+} allows this paramagnetic contrast agent to enter activated neurons, while the lack of effective cellular mechanisms to export Mn^{2+} results in accumulated concentrations over time in brain regions exposed to Mn^{2+} [2]. Past comparison of MEMRI to BOLD and CBF fMRI in the rat somatosensory cortex showed excellent agreement, but required intra-arterial injection of mannitol to break the blood-brain-barrier [3]. A recent report demonstrated that simple subcutaneous injection of $MnCl_2$ resulted in MEMRI enhancement of many mouse brain regions known to be highly active under normal conditions, including the auditory midbrain [4]. We tested the sensitivity of MEMRI, with IP injection of $MnCl_2$, to detect auditory-evoked activity in the mouse brain.

Methods

Mice were maintained according to protocols approved by the Institutional Animal Care and Use Committee at New York University School of Medicine. Swiss-Webster mice were injected IP with 0.2 mM/kg body weight of $MnCl_2$ solution in water. This dose was found to be safe, yielding no obvious toxicity in all mice tested. Auditory stimulation consisted of sinusoidally-modulated white noise pulses, 4 pulses/s. Mice were alternately subjected to 2-h of auditory stimulation, followed by 1.5-h of MRI (0.5-h preparation, 1-h acquisition time) over the course of the day, resulting in 3-D image data acquired at 3, 6.5, and 10-h post Mn-injection. Mice were anesthetized with isoflurane (1-1.5% in air) during MRI, but were awake and behaving normally during auditory stimulation intervals. Control mice were deafened at least one week before MEMRI, by puncturing the tympanic membrane and surgically removing the malleus, and were injected and imaged exactly the same as the stimulated mice. MRI was performed on a 7-T SMIS console interfaced to a horizontal magnet and 250-mT/m actively shielded gradients (Magnex) with a custom holder and 22-mm (ID) saddle coil, using a 3-D T1-weighted spin echo sequence (TE/TR = 8/300 ms) with spatial resolution of $100\mu m \times 100\mu m \times 500\mu m$ (total imaging time = 1-h).

Results and Conclusion

Stimulated mice showed MEMRI enhancement in the dorsal midbrain (inferior colliculus, IC), compared to pre-Mn-injection images from the same mice (Fig.1). This enhancement was obvious from the earliest post-injection images (3-h), and images of the IC were markedly different from those acquired in deaf controls at all post-injection time points (Fig. 1). ROI analysis, normalized to pre-injection images, showed a statistically significant 10-15% MEMRI enhancement in the IC ($p < 0.05$ at all post-injection times) compared to deaf controls, that was not observed in the (non-auditory) caudate putamen (CPu), measured in the same stimulated mice (Fig. 2). Higher auditory regions such as the medial geniculate nucleus (MGN) and auditory cortex (AC) also demonstrated MEMRI enhancement (~5%) that was time-dependent (maximum enhancement at 3-h in AC, and at 6.5-h in MGN), but the differences compared to deaf control mice, did not reach statistical significance (data not shown). We conclude that this easily-implemented MEMRI protocol enables the detection and quantification of auditory-evoked neural activity in the mouse midbrain.

Acknowledgement This research was supported by NIH grant R01NS38461.

References

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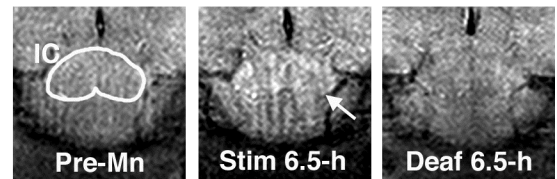


Fig 1: Enhancement (arrow) was observed in the IC (outlined, left panel) on horizontal T1-weighted MRIs of all Mn-injected, stimulated mice (middle), but not on pre-Mn images (left) or on images of deaf control mice (right). The example shows the result after 6.5-h.

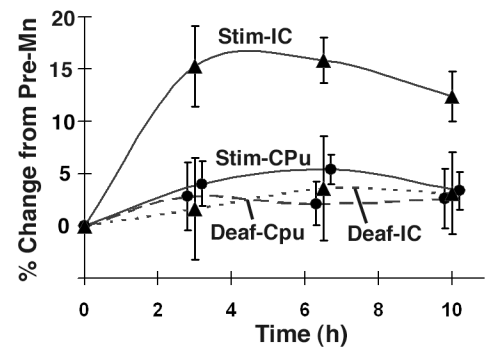


Fig 2: Quantitative ROI analysis of MEMRI enhancement in the IC and CPu, normalized to Pre-Mn injection images (stimulated, Stim N=6; controls, Deaf N=6).