Detection of Blood-Brain Barrier Disruption in Rat Brain After Osmotic Shock Using Manganese-Enhanced Magnetic Resonance Imaging (MEMRI)

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Introduction. MEMRI experiments[1-3] employ the intracellular uptake of Mn^{2+} through voltage-gated calcium channels to shorten the T_1 relaxation time of intracellular water resulting in localized increases in T_1-weighted MRI signal intensity which correspond to regions of neuronal activation.[1] These experiments rely on disruption of the blood brain barrier (BBB) by osmotic shock to deliver a significant concentration of Mn^{2+} into the extracellular space prior to the neuronal stimulus. In order to optimize the contrast, the distribution of extracellular Mn^{2+} should be homogeneous across the region of interest with little or no initial MR signal enhancement prior to the neuronal stimulus. Here we show that the timing of Mn^{2+} administration relative to BBB opening, as well as the method of Mn^{2+} delivery, are important factors in achieving this goal. Regions of T_1-contrast enhancement were spatially correlated with areas of Evans Blue (EB) dye uptake after hyperosmotic BBB disruption. A significant increase in T_1-weighted signal enhancement was observed following internal-carotid-artery infusion of Mn^{2+} two minutes after hyperosmotic BBB opening; indicating a significant role for MEMRI in visualizing BBB disruption under these circumstances. However, this enhancement would also obscure T_1-weighted signal increases arising from subsequent neuronal stimuli; thus mitigating against using this particular administration protocol for such studies.

Methods. Four male Sprague-Dawley rats weighing 280-350 g were anesthetized with 2.0 % isoflurane mixed with breathing-quality air. 25% D-mannitol solution (5 ml/kg; Sigma) was injected into the internal carotid artery over 50 seconds. Prior to each injection, the common carotid, external carotid and pterygopalatine artery were ligated. Two minutes after the mannitol injection was completed manganese chloride ($MnCl_2$, MW = 198; Sigma), dissolved to 30 mM in isotonic saline, and EB (Sigma), dissolved to 2% in manganese chloride solution (2 ml/kg), was infused into the internal carotid artery at 4.5 ml/hr. MR images were acquired using a Bruker Biospin 2.0T/45 cm imaging spectrometer operating at 85.56 MHz for ^1H and equipped with ±20G/cm self-shielded gradients. T_1-weighted MRI was performed with the following acquisition parameters: TR/TE = 300/7 msec, FOV = 3 cm x 3 cm, matrix = 128x128, six 2-mm slices with NEX = 8.

Results. Figure 1A shows signal enhancement from T_1-weighted MEMRIs following administration of 30 mM $MnCl_2$. Figure 1B shows EB staining in the corresponding histological brain slices. The average regional enhancement was ~250% of that in the homologous contralateral region (e.g., see ROIs in the top MRI slice of Fig. 1A). As seen in Fig. 1 EB histological staining correlates well with areas of increased T_1-contrast in the corresponding MRI slices. For this particular animal, the effect of Mn^{2+} on T_2 relaxation shows the importance of T_1-weighted imaging with a short TE to minimize signal loss due to T_2 and T_2^* as Figure 2 demonstrates.[4] Figure 2 shows images acquired with TE = 7 ms (Fig. 2A and Fig. 1A top row) and 20 ms (Fig. 2B). The negative contrast shown in Fig. 2B is likely due to higher concentrations of Mn^{2+} in the respective brain regions. Also, as seen in Fig. 1, EB histological staining is darker in the regions corresponding to higher $[Mn^{2+}]$ suggesting more extensive BBB opening. Figure 2C shows the MR signal decline as a function of TE in the cortical ROI shown in Fig. 1A.

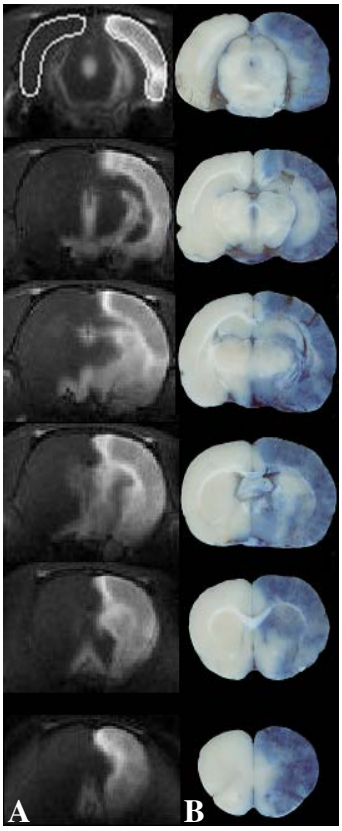


Figure 1: T1-weighted MRIs **(A)** and corresponding EB-stained histological slices **(B)**. 30 mM $MnCl_2$/2% EB was administered 2 min after osmotically-induced BBB opening. Areas of MRI signal enhancement correlate well with regions of BBB opening on EB-stained slices. Top MRI slice in **(A)** shows the ROIs used for enhancement calculations.

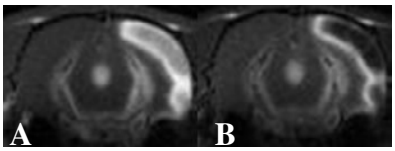
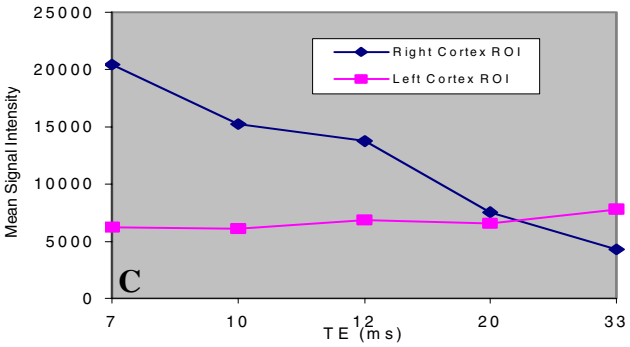


Figure 2: T_1-weighted MEMRI following administration of 30 mM $MnCl_2$. Images acquired with TR = 300 and TE = 7 ms **(A)** and 20 ms **(B)**. Signal loss due to T_2 and T_2^* effects on the image suggest high concentrations of intracellular manganese.[4] **(C)** MR signal intensity in cortical ROIs as a function of TE (TR = 300 ms).



Discussion. MEMRI experiments for visualizing the brain response to neuronal stimulation rely on disruption of the blood brain barrier (BBB) by osmotic shock to deliver a significant concentration of Mn^{2+} into the extracellular space prior to the neuronal stimulus. However, there should be little or no initial MR signal enhancement prior to the neuronal stimulus in order to achieve optimal T_1-contrast enhancement. Although the administration protocol used in this study has been employed by others,[3] the time period between BBB opening and Mn^{2+} administration was longer (approximately 15 minutes) than that used in this study (two minutes). Using the current administration protocol, significant initial T_1-enhancement would obscure T_1-contrast changes arising from any subsequent neuronal stimulus; thus precluding the use of this particular dosing regimen for such studies. However, this Mn^{2+} administration method is quite effective in visualizing the region of BBB opening under these circumstances. The strong T_1-contrast enhancement observed in these experiments suggests a large accumulation of intracellular Mn^{2+} immediately following osmotically-induced BBB opening; assuming that the mechanism of contrast change is similar to that observed following neuronal stimulation or for white-matter-tract tracing. However, because of the dramatic changes in cell volume that occur under these circumstances, Mn^{2+} sequestered in the extracellular space may also be a contributing factor. In spite of this uncertainty, we observed (data not shown) that regions of T_1 contrast did not change significantly over time (3 hours) with respect to 1) spatial position, 2) region size, or 3) level of enhancement. These observations are consistent with the intracellular uptake of Mn^{2+}, where signal-enhancement levels have been shown to be maintained for several hours because of intracellular Mn^{2+} sequestration following neuronal stimulation.[1] However, an extracellular contribution to the T_1-contrast enhancement cannot be ruled out, since closure of the BBB following osmotic shock could also trap a significant concentration of Mn^{2+} in the extracellular space, preventing its subsequent washout over time. Finally, this administration protocol may be a useful alternative for imaging experimental BBB disruption.

References. [1] Lin et al. *Mag. Reson. Med.* **38**: 378-88 (1997). [2] Henning et al. *Mag. Reson. Med.* **53**: 851-857 (2005). [3] Aoki et al. *Mag. Reson. Med.* **50**:7-12 (2003). [4] Silva et al. *NMR in Biomed.* **17**: 532-543 (2004).