Excitotoxic and physiological stimulations provide similar signal enhancement on manganese-enhanced MRI

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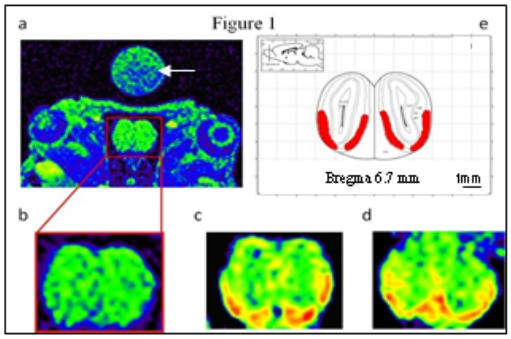
Introduction: Manganese (Mn^{2+}) is a paramagnetic contrast agent that can be easily detected in T1-weighted MR images. During neuronal physiological activation, extracellular Mn^{2+} (an analogue of calcium - Ca^{2+}) can enter neurons through voltage-gated Ca^{2+} channels (VGCC, **1**). Intracellular Mn^{2+} can be transported along axons and across synapses thus allowing for tract tracing as shown in the olfactory (**2**) and visual systems (**3**). These applications of Manganese-Enhanced Magnetic Resonance Imaging (MEMRI) can facilitate the evaluation of functional circuits in normal animals or in animals with developmental alterations (**4**). In addition, functional MEMRI can help to evaluate neuronal dysfunction during neurodegenerative diseases such as in Alzheimer's (**5**) or Parkinson's (**6**) disease and to study animal models of these pathologies. Excitotoxic events are associated with these diseases via a hyperactivation of NMDA receptors (NMDAR) that leads to an overload of Ca^{2+} inside the cells. In animal models of these diseases, an increased entry of Mn^{2+} inside the cells might thus be related to excitotoxic events rather than to functional activation. This might lead to misinterpretations of MEMRI images. To compare MEMRI results between physiological activation and excitotoxicity in the same system, we evaluated MEMRI signal intensity in odor-evoked activation versus quinolinate (an agonist of NMDAR) lesions in the olfactory bulb of rats.

Materials and methods: MR images of the olfactory bulbs were recorded using a 3T spectrometer (MAGNETON Trio MR, Siemens) and phase array coils dedicated to human elbow MRI. T1-weighted 3D images were recorded using gradient echo sequences (Resolution=250x250x500 μm^3, FOV=32x32x32 mm^3, Matrix=128x128x64, TR/TE=11/4 ms, Alpha= 9°, NA= 32, Acquisition time= 37 min). Sprague Dawley rats were divided into 4 groups (Table 1): control animals (n=6); animals exposed to functional odor stimulation (odor, n=6); animals with quinolinate lesions (bilateral injection of 1 µl of quinolinate at 90 mM) in the olfactory bulb (quino, n=7); and animals which received saline injections (sham, n=7).

		DAY 1	DAY 2	DAY 3		
Physiological condition	Control	baseline MRI (Fig. 1a, 1b)	-	MnCl2	-	MEMRI 1, 2, 3
	Odor	baseline MRI	-	MnCl2	odor	MEMRI 1, 2, 3 (Fig. 1c)
Pathological condition	Sham	baseline MRI	Surgery, saline	MnCl2	-	MEMRI 1,2,3
	Quino	baseline MRI	Surgery, quinolinate	MnCl2	-	MEMRI 1, 2, 3 (Fig. 1d)

Table 1. Experimental procedure. MEMRI 1: 90 min post-MnCl2; MEMRI 2: 150 min post MnCl2; MEMRI 3: 200 min post MnCl2.

For the MEMRI, rats were lightly anesthetized with isoflurane and 5 µl of $MnCl_2$ solution (1 M) was injected into the naris with a catheter. After recovering from anaesthesia, animals from the odor stimulation group were exposed to amyl acetate for 20 minutes using 30s on/off pulses. Animals from other groups were not exposed to any odor. All animals were imaged at three time points (90, 150, and 200 mins) post-manganese administration and were anaesthetized (ketamine+medetomidine) during MRI acquisition. The signal was evaluated within regions of interest (ROI) located approximately 2 mm from the rostral edge of the olfactory bulbs, in areas corresponding to superficial layers of the olfactory bulbs where the functional units (olfactory glomeruli) are located (Fig. 1). ROIs were first identified on the 200 min scan, then they were reported on the pre-manganese scans, and on 90 and 150 mins post-manganese scans. Signals from olfactory ROIs were first normalized using a reference signal from a water tube located on the top of the animal nose (Fig. 1a). Then, MEMRI-related signal enhancement for each olfactory ROI was calculated by dividing normalized signals from the MRI recorded after manganese injection by normalized signal evaluated on pre-manganese scans, i.e. using each animal as its own control. Histological evaluation of the brain lesions was carried out 1 day and 2 weeks after quinolinate injections and did not show any massive cell loss (data not shown).



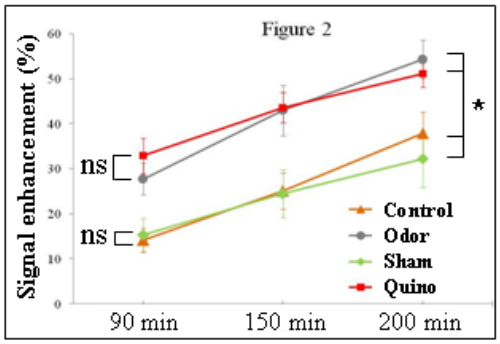


Figure 1. T1-weighted MR images of rat olfactory bulbs. (a): in each rat, signals were normalized relative to the water tube (arrow) above the head. (a, b): MRI recorded before MnCl2 injection. (c, d): MRI recorded 200 min post-injection in an odor-stimulated rat (c) versus in a quinolinate-injected rat (d). Enhanced signals are pseudo-colored in red and correspond to glomerular areas. (e): Regions of interest where signal enhancement was computed (anatomical reference: Paxinos and Watson atlas, scale bar represents 1 mm).

Figure 2. Time-course of MEMRI signal enhancement (mean ± SEM). ns: non significative. *: $p<0.05$.

Results and Discussion: In all groups, Mn^{2+} administration led to T1-signal changes at the rostral level of the olfactory bulbs (Figs. 1, 2) in regions corresponding to olfactory glomeruli, i.e. the functional modules receiving the olfactory neurons projections (**8**). These signal changes indicate that, Mn^{2+} was actually transported into the olfactory glomeruli and confirms Pautler and Koretsky's report (**7**). A graph of the time course of the percentage of signal change for each group is presented in Fig. 2. The signal increased in a similar way in the "control" and "sham" groups ($F(1, 12)=0.074$; ns). However, the signal increased further in the "odor" and "quino" groups compared to "control" and "sham" groups (ANOVA tests: $ps<0.05$). Interestingly, there was no difference in signal intensity between the "odor" and "quino" groups ($F(1, 9)=0.002$; ns).

The increase in MEMRI signal in the olfactory bulb of all groups can be explained by the following events: Mn^{2+} entered the spontaneously active olfactory neurons in the olfactory epithelium via VGCC; it was then transported and released by the synaptic terminals of these cells in the olfactory glomeruli, and there, Mn^{2+} entered the postsynaptic cells via VGCC. During odor stimulation, the olfactory neurons detected the odorant molecules and were specifically activated above their spontaneous activity producing further postsynaptic activity and thus an increased MEMRI signal in the olfactory bulb. The main result from our study is the increased MEMRI signal in the "quino" group as compared to the "control" and "sham" groups. Very interestingly, in this case the local injection of quinolinate activated NMDAR and in cascade VGCC (**9**), thus allowing a large entry of Mn^{2+} in postsynaptic cells. This influx of Mn^{2+} mimicked functional activation on MEMRI images although it was mainly related to cell suffering..

In conclusion, our data suggest that manganese produces similar enhancement of MEMRI contrast during both physiological stimulation and excitotoxic activation. This finding suggests that in animal models of neurodegenerative disease, studies based on MEMRI should be conducted with caution because the loss of physiological activation can be compensated by excitotoxic-related signal enhancement.

References 1-Koretsky, Silva. NMR Biomed. 2004, 2-Pautler et al. Magn Reson Med. 1998, 3-Watanabe et al. NMR Biomed. 2004, 4-Yu et al. Nat Neurosci. 2005, 5-Smith et al. Neuroimage 2007, 6-Pelled et al. J Magn Reson Imaging 2007, 7-Pautler, Koretsky. Neuroimage 2002, 8-Lin, Koretsky. Magn Reson Med. 1997, 9-Shepherd and Greer, Oxford University Press, New York 1998.