

# Manganese Enhanced MRI of Olfactory Pathway in Developing Rats

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## Introduction

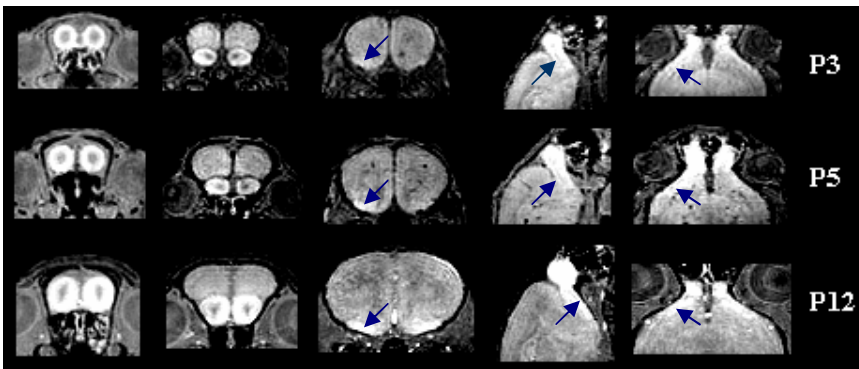
Manganese enhanced MRI (MEMRI) is a powerful tool for activity-dependent trans-synaptic tract tracing with high spatial resolution. Previous work has shown that the olfactory pathway can be traced with MEMRI in rodents after administration of manganese to the nasal epithelium [1]. This study extends this work to neonatal rats, in order to assess the feasibility of using MEMRI to study changes in the function of the well-characterized rat olfactory system during postnatal development.

## Materials and Methods

Rat pups of different age groups were anesthetized and 5 $\mu$ L of 1M MnCl<sub>2</sub> was injected in both the nostrils. The animals were returned to the home cage and were imaged 21-27 hours post-injection. All imaging experiments were performed on Bruker 9.4T using 35 mm volume coil. T1 weighted images were obtained using a 3D FLASH sequence with the following parameters: FOV = 1.92  $\times$  1.92  $\times$  2.56 cm<sup>3</sup>, acquisition matrix size = 96  $\times$  96  $\times$  128, reconstruction matrix size = 128  $\times$  128  $\times$  128, TR = 70 ms, TE = 4 ms, NEX = 2, imaging time = 21 minutes. The following 3 groups were used for statistical comparisons: postnatal day 3 (P3), P5, and P10-P12 (n = 4 for each group). Regions of interests covering olfactory bulb (OB), anterior olfactory nucleus (AON) and olfactory cortex (OC) were drawn manually. Percentage enhancement in each region was calculated using neighboring nearby non-enhanced neocortical area as a reference. Two data points per animal (one data point for each side) were obtained, resulting in 8 data points per group. Two-tailed t-tests were performed between the aforementioned groups of animals. In addition, a variant of Manganese Synaptic Transfer Index (MSTI) [2] was calculated for all groups using neighboring non-enhanced region for normalization instead of muscle. To do that, mean intensities in OB, OC and AON were normalized to the neighboring cortical regions. Then, the following ratios were obtained: 1) Ratio of normalized intensity in AON to normalized intensity in OB 2) Ratio of normalized intensity in OC normalized intensity in OB.

## Results and Discussion

The olfactory system was successfully imaged with MEMRI at all ages. Enhancement of OB and AON was readily apparent in all rat pups, with the OC detectable but less apparent in the youngest rats (Fig.1). Tracts originating from olfactory bulb were observed in images of animals of all age groups. Results summarized in Table 1 suggest increase in percentage enhancement with age at least between postnatal days 3 and 12. The p-values were considerably smaller for comparisons between animals with larger age difference (Table 2), although not statistically significant due to the small number of pups imaged to date. Average ratios for AON/OB and OC/OB were 0.95  $\pm$  0.04 and 0.95  $\pm$  0.07 for P3; 0.93  $\pm$  0.06 and 0.93  $\pm$  0.06 for P5, 0.95  $\pm$  0.03 and 0.92  $\pm$  0.06 for P10. No significant age-dependent changes were observed in these ratios (p-value>0.3 for all comparisons).



**Figure 1:** Columns 1-3 (from left to right) show coronal slices covering OB, AON and OC respectively. Columns 4-5 show tract tracing in sagittal and horizontal slices respectively.

The results of this experiment indicate that enhancement of the olfactory system can be successfully detected with MEMRI in rats as early as P3, creating the possibility of using MEMRI to study developmental disruptions such as olfactory deprivation. Ongoing experiments will increase the group sizes and improve the statistical significance of the results presented in this abstract. Also, we intend to make comparisons between animals with ages varying over a wider range. We have acquired DTI images at different ages (data not shown) and we intend to use information obtained from DTI images in conjunction with MEMRI to characterize normal and disrupted functional development of the rat olfactory system.

## References

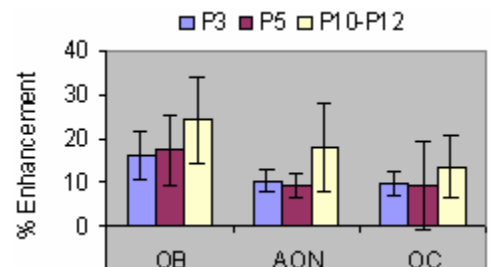
- [1] Pautler, RG et al. Magn. Reson. Med. 1998; 40(5):740-748.  
 [2] Smith, K. et al. Proc. Intl. Soc. Magn. Reson. Med. 14 (2006): 224.

Age	% Enhancement		
	OB	AON	OC
P3	16.09 $\pm$ 5.55	10.21 $\pm$ 2.63	9.87 $\pm$ 2.82
P5	17.25 $\pm$ 8.02	9.12 $\pm$ 2.77	9.18 $\pm$ 9.98
P10-P12	23.93 $\pm$ 10.00	17.97 $\pm$ 10.00	13.57 $\pm$ 7.39

**Table 1:** Percentage enhancement in different brain regions in rat pups of different ages

Comparison	p-Value		
	OB	AON	OC
P3 vs P5	0.76	0.40	0.86
P3 vs P10-P12	0.11	0.06	0.17

**Table 2:** p-values for comparisons of percentage enhancement between pups of different ages



**Figure 2:** Graphical presentation of the data summarized in table 1