# 3D Statistical Mapping of Odor Induced Differences in Manganese Uptake in the Mouse Olfactory System

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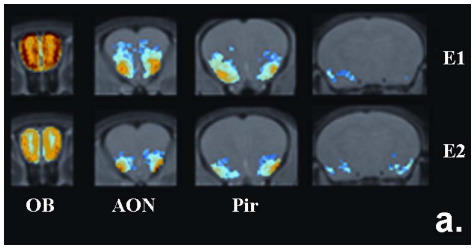
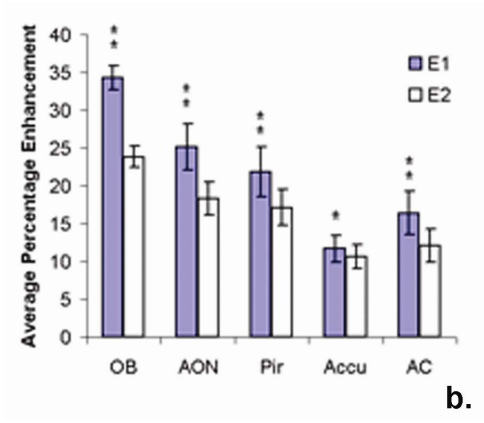
**Introduction:** Manganese enhanced MRI (MEMRI) is a powerful tool for activity-dependent trans-synaptic tract tracing with high spatial resolution. Previous work has shown that the olfactory pathway can be traced with MEMRI in rodents after administration of $Mn^{2+}$ to the nasal epithelium [1] and stimulation-induced differences in enhancement can be observed in the olfactory bulb [2]. 3D statistical maps can be created to quantify enhancement [3]. Pautler et al [2] examined effects of olfactory stimulation during early stages of $Mn^{2+}$ uptake, which did not allow sufficient time for tract tracing. In this work, we quantify the effect of repeated propanol exposure on $Mn^{2+}$ uptake throughout the olfactory tract in adult mice after nasal $MnCl_2$ administration.

**Materials and Methods:** 12 adult mice were imaged prior to $Mn^{2+}$ administration on Bruker 9.4T scanner using a 3D FLASH sequence with following parameters: Flip angle = 30°, FOV = 1.92 ×1.92 × 1.92 $cm^3$, acquisition matrix size = 96 × 96 × 96, reconstruction matrix size = 128 × 128 × 128, TR = 70 ms, TE = 4 ms, NEX = 2, imaging time = 21 minutes. The animals were divided into two groups with n = 6. The mice in the first group (E1) were anesthetized with 5% isoflurane and were injected nasally with 5µl of 1M $MnCl_2$ solution with a Hamilton syringe bilaterally. The animals were then returned to their home cage and were imaged approximately 42 hours post $Mn^{2+}$ exposure. The mice in the second group (E2) were anesthetized and injected with $MnCl_2$ solution as described above. After recovery from anesthesia, they were exposed to propanol for 10 minutes followed by five minutes of room air. This exposure was repeated three times. The following day these mice were exposed to propanol every four hours, each exposure lasting 15 minutes. The animals were imaged approximately 42 hours post $Mn^{2+}$ exposure using the same parameters as before. All the images were registered and normalized to one of the control images (acquired prior to the exposure). Two-tailed unpaired was performed on voxel-by-voxel basis between the control group and each of the treated groups. Significantly enhanced voxels ($p < 0.01$) were clustered and maps were created, displaying percentage enhancement for the significantly enhanced voxels. Mean percentage enhancement in different ROIs was compared between E1 and E2.

**Results and Discussion:** Tracts originating from the olfactory bulb were observed in the maps obtained from both of the groups. Statistically significant enhancement was observed in olfactory bulb (OB), anterior olfactory nucleus (AON), piriform cortex (Pir), olfactory tubercle (OT), nucleus accumbens (Accu) and basolateral amygdala (BLA) for both groups (fig a).




A larger enhanced area and higher percentage enhancement in selected ROIs were observed in E1 as compared with E2 (fig a, b). Although greater enhancement was observed for E1 throughout the olfactory tract, it does not necessarily reflect greater neural activity at all levels, because increased uptake in bulb will result in increase in amount of $Mn^{2+}$ available for transportation to rest of the tract. Our results seem to contradict previous reports of regions of increased enhancement due to olfactory stimulation [2]. However, the reduction in uptake can possibly be attributed to presynaptic inhibition of olfactory sensory neurons due to 'short axons' cells [5]. Since propanol activates fewer glomeruli as compared with amyl acetate [4], smaller area in the bulb is activated as compared with amyl acetate. Subsequently, larger area is expected to be inhibited, possibly to decrease the overall 'noise' of olfactory information and to increase neural specificity of the response to the odor. This can explain the overall reduction in $Mn^{2+}$ uptake. Also, it should be noted that the time-scales for our study and that presented in [2] are very different.




Since the mice in group E1 were not removed from the home cage after the injection and before imaging as E2 mice were, the exposure to a novel environment can be a possible confounding factor. Future experiments will eliminate that issue.

This work, along with previous studies, suggests that MEMRI can be a useful tool for detecting stimulus-induced changes in activity throughout a sensory neural pathway. The potential to detect activity changes in response to behaviorally relevant stimuli suggests that statistical group comparisons with MEMRI may be sensitive enough to provide insight into changes that occur during learning or development.

**References:** [1] Pautler, RG et al. Magn. Reson. Med. 1998; 40(5):740-748 [2] Pautler, RG and Koretsky, AP. Neuroimage 2001; 16:441-448 [3] Cross, DJ et al. Neuroimage 2004; 23(4):1326-1335 [4] Johnson, BA et al. J. Comp. Neurol. 2000; 422(4):496-509 [5] Aungst, JL et al. Nature 2003; 426:623-629