Detection of olfaction induced activation in the brain after systemic manganese infusion

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Introduction
In most functional MEMRI-studies the manganese has been delivered to the brain either as a local stereotactical injection or as a systemic injection combined with D-mannitol administration to break the blood-brain barrier. A study by Yu et al. (2005) indicated that functional MEMRI would be possible in the auditory brainstem nuclei (cochlear nucleus and inferior colliculus) and thalamus also by using systemic manganese administration without the disruption of the blood-brain barrier. The aim of this study was to test the same approach in the olfactory system because it is easy to stimulate and manganese reaches the olfactory bulb quickly and in high concentrations after systemic administration even with an intact blood-brain barrier.

Materials and methods
Adult male Wistar rats were studied under urethane anaesthesia. Animals were divided into two study groups: animals with manganese infusion and olfactory stimulation (n=5), and control animals with manganese infusion but no olfactory stimulation (n=4). In addition a control animal underwent the experiment with olfactory activation without manganese infusion. After acquiring control images, olfactory stimulation was started and MnCl (60 mg/kg in 45 minutes) was infused into the femoral vein. In the olfactory stimulation we used 18 different scents with 5 min stimulation periods to avoid adaptation induced lowering of activation in the brain. The stimuli were carried to the face mask of animals in a gas mixture of 70 % N_2O / 30 % O_2.

MRI was performed in a 4.7T Varian Inova MRI system using an actively-decoupled volume coil-quadrature surface coil pair. Data for T1-maps were collected using inversion recovery fast spin-echo sequence (TI's 100, 300, 700 and 1500 ms, TR 6 s, 16 echoes, echo spacing 10 ms, FOV 35 x 35 mm thk 1 mm, resolution of 256 x 128). Two imaging slices were imaged located in the middle of olfactory bulb or olfactory cortex. Absolute manganese concentrations in blood, CSF and brain tissues were determined by fAAS (furnace atomic absorption spectroscopy) and dfAAS (direct flame absorption spectroscopy) methods from samples taken at different time points during and after a systemic manganese infusion (n=3, control with saline infusion n=1).

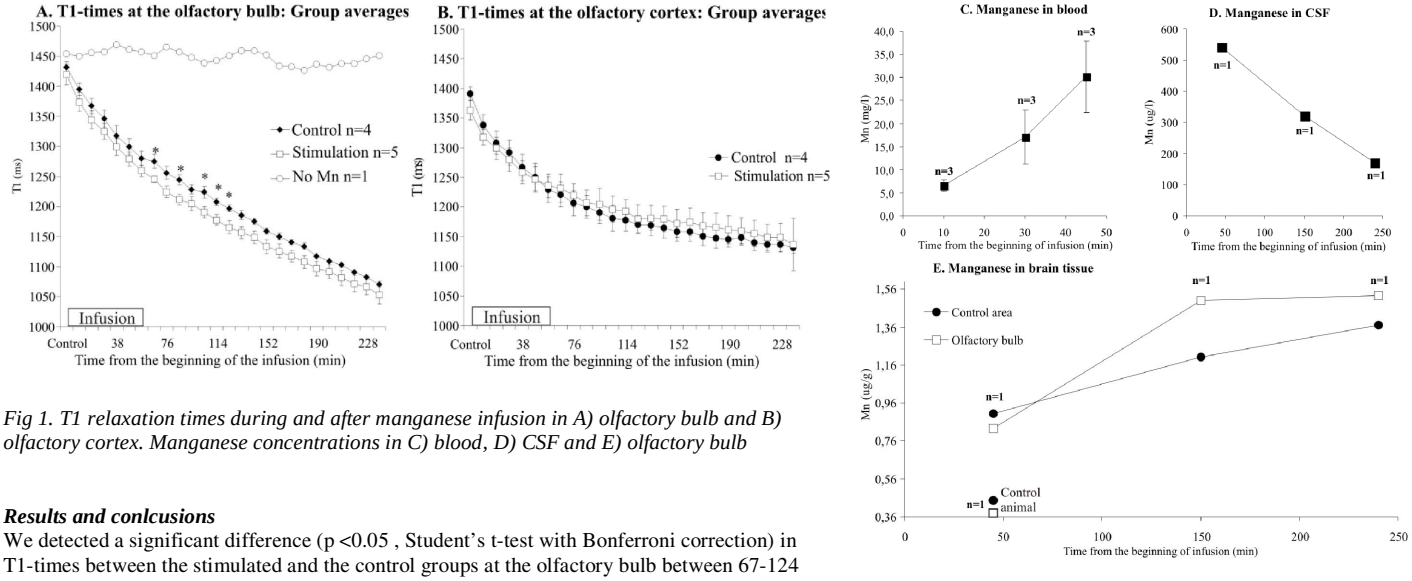

Fig 1. T1 relaxation times during and after manganese infusion in A) olfactory bulb and B) olfactory cortex. Manganese concentrations in C) blood, D) CSF and E) olfactory bulb

Results and conlcusions
We detected a significant difference ($p < 0.05$, Student's t-test with Bonferroni correction) in T1-times between the stimulated and the control groups at the olfactory bulb between 67-124 min. In olfactory cortex, accumulation of Mn as indicated by T1 decrease, was slower than in the olfactory bulb and no significant difference was detected between control and stimulated group. AAS analysis showed increasing Mn concentration in blood during infusion, rapid transfer to the CSF and decrease of Mn concentration with the rate of -1.8 µg/l/min after the end of infusion and accumulation of tissue Mn during and after infusion.

Conclusions
The olfactory stimulation increased the cellular manganese uptake, leading to higher local manganese concentrations and shorter T1-times compared to control group in the olfactory bulb. The difference in T1-times between the groups was significant after 67 min from onset of MnCl infusion, and remained for 1 h when the Mn concentration in CSF was between 542 and 322 µg /l. After this time the difference in manganese accumulation in the tissue between the groups was no longer maintained, likely because the manganese levels in the CSF started to limit the uptake into the tissue. In the olfactory cortex the extracellular Mn concentration seems to be the limiting factor for Mn uptake and thus the activation dependent accumulation was not detected. It appears that activation dependent MEMRI with systemic Mn administration with intact BBB is possible only in specific brain regions that have naturally more permeable blood-brain barrier.

References
Yu X, Wadghiri YZ, Sanes DH & Turnbull DH, 2005. In vivo auditory brain mapping in mice with Mn-enhanced MRI. Nat Neurosci 8: 961-968.