# Dynamics of Mn transport in the mesolimbic system reveal neural projections from the Nucleus Accumbens in vivo

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**Introduction:** The Nucleus Accumbens (NAc) plays a fundamental role in the neural reward circuit and studies on altered-genotype mice have linked individual chemical components with behavioral consequences of drug abuse [1,2]. An improved understanding of this circuit and the neurophysiology of addiction may lead to more effective treatments and eventually to cures and preventive measures. Previous studies show that Mn transport and accumulation are useful indicators of specific neural structures, activation and, consequently, function [3,4,5,6,7]**.** The aim of this work was to study the feasibility of MEMRI to map mesolimbic neuronal circuitry, activation and anatomy in studies of addiction in vivo. We monitored the dynamics of Mn transport along the neuronal projections from the NAc using $T_1$-w MRI after stereotaxic injections of $MnCl_2$. Whole brain voxel based morphometric analysis and SPM enabled the unbiased observation of the spatiotemporal connectivity in vivo in the mesolimbic system, an important dopaminergic pathway in the mammalian brain.

**Materials and methods:** All animal experiments conformed to institutional guidelines. Wild type female C57Bl6 mice (n=6) were anesthetized with isoflurane (1.5% in $O_2$) and placed in a stereotaxic frame. A volume of 3 nL $MnCl_2$ [1.2M] was injected in the NAc (1.4 mm ML, 0.74 mm AP and -4.8 mm DV [8]) using a glass pipette (inner diameter=15 um). $T_1$-w 3D MR images were acquired with a 3D RARE sequence before, 10 min, 3.5 hr, 7.5 hr, and 21.5 hr after injection with the following parameters: TR=250 ms, TE=6 ms, RARE factor=4, NA=4, matrix=160x128x78, 100 µm isotropic voxel size and acquisition time of 42 min. A custom made mouse head birdcage r.f. coil was used and all experiments were performed at 11.7 T. The acquired 3D MR data were masked using BrainSuite, linearly aligned using a 12-parameter full-affine transformation [9] creating an unbiased template, and non-linearly aligned [10] to this template to warp all the data in the same 3D space at every time point. Images were blurred with a Gaussian kernel and statistical parametric maps were created in SPM5 using a paired student's t-test with FDR correction to correlate significant increases in intensity with underlying anatomy. A value of $p < 0.05$ was considered significant.

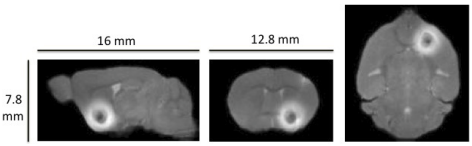


**Fig. 1: Average of 30 min post injection T1w images after linear and non-linear alignment (n=6).**

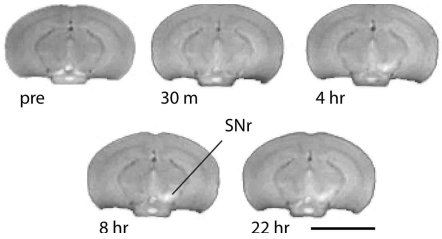


**Fig. 3: Raw data of Mn induced signal intensity (bar = 5 mm)**

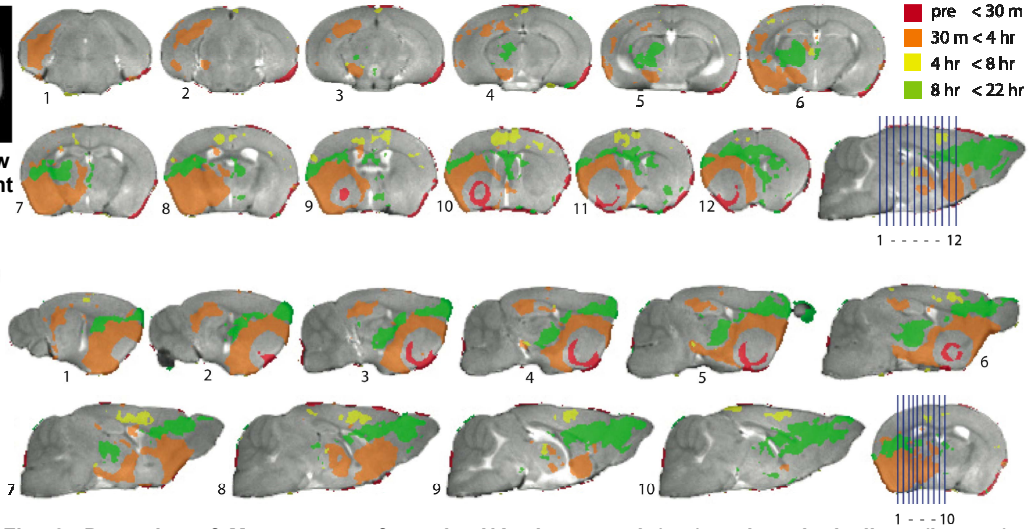


**Fig. 2: Dynamics of Mn transport from the NAc in coronal (top) and sagittal slices (bottom). Averaged images of registered pre injection data are overlapped by color-coded parametric maps, displaying significant intensity changes (p<0.05) over time. Red indicates the significant intensity change between pre injection and 30 min after injection, orange between 30 min and 4 hr after injection, yellow between 4 hr and 8 hr and green between 8 hr and 22 hr after injection.**

**Results and discussion:** All injections were located in the NAc (fig 1) and were performed within a radius of 0.15 mm from each other. Mn transport was visualized by MEMRI and SPM for an unbiased assessment (fig.2). As shown in fig 2, Mn transport in normal mice delineates most of the expected connections of the mesolimbic system [11]. In 4 hr, Mn ions were transported to the substantia nigra (SNr), ventral tegmental area (VTA), the dorsal medial thalamus (DMT) and the hippocampus. After 4 hr, isotropic diffusion of Mn ions impaired visualization of active transport to the ventral pallidum (VP) and amygdala (AMG). 8 hr after injection, Mn accumulates and progresses further to the DMT, PFC and SNr. An afferent connection exists between the NAc and the prefrontal cortex (PFC) and an efferent one via the ventral pallidum (VP) and the DMT. In the afferents to the hippocampus, DMT and VTA, contrast was enhanced 4 hr post injection, while Mn was observed in the PFC after 8 hr. After 22 hr, Mn induced intensity is seen in the thalamus, caudate putamen, and prefrontal cortex and extends to the olfactory bulb. Remarkably, there is no significant signal change in SNr between 8 and 22 hr (fig 2 and 3). Since the SNr is known to accumulate Mn [12, 13] this may indicate compartmentalization of Mn.

**Conclusion**: Using MEMRI and SPM, the afferent and efferent neural projections from the NAc were visualized in vivo, offering insights in the dynamical behavior of different brain areas of the reward circuitry. These findings allow for future studies involving inhibition or stimulation of the reward system and the use of genetic models, to investigate differences in neural activity and anatomy in the mesolimbic system.

**References**: [1] H. Ishiguro, *Neuropsychoph*, 31 (2006) [2] I. Sora, *PNAS*, 98 (2001) 5300-05 [3] H. Lu, *PNAS*,7 (2007) 2489-94 [4] E.L.Bearer, *NeuroImage*, 4( 2009) 1091-1104 [5] R. Pautler, *Neuroimage,* 16 (2002) 740-8 [6] A.F. Silva, *NMR Biomed*, 17 (2004) 532–543 [7] R. Pautler, *MRM,* 40(1998)740-48 [8] G. Paxinos, Academic Press (2001) [9] R. P. Woods, *JCAT,* 22 (1998a-b) 139-65 [10] D. Rex, *Neuroimage*, 19 (2003) 1033-48 [11] E. J. Nestler, *Nat. Rev. Neuroscience*, 2 (2001) 119-28 [12] W. N. Sloot, *Brain Res*, 657 (1994) 124-32 [13] M. Aschner, *EHP*, 108 (2000) 429-32