Rat brain connectivities after stroke: combined application of diffusion tensor MRI (DTI), manganese enhanced MRI (MEMRI) and functional MRI (fMRI)

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Introduction: We have shown earlier that there is strong inter-individual variability of chances for functional recovery from stroke in the rat, despite closely similar lesion volumes. Therefore, the hypothesis is that a morphological correlate for the functional deficit and/or functional recovery may be the thalamo-cortical connectivity on the ischemic hemisphere. The aims of the present study were the establishment and application of an optimized experimental protocol to investigate this hypothesis in longitudinal studies in individuals. High spatial and temporal resolution DTI and MEMRI for monitoring intactness of neuronal tracts in rat brain were combined with repetitive fMRI results on the same individuals. The investigation was performed to investigate cortico-thalamic connectivity, in rat brain during 2 months after stroke.

Methods: *Animal model:* Stroke was induced in 6 male Wistar rats by transient (1h) occlusion of the right middle cerebral artery. For all surgery and during MEMRI and DTI animals were kept under halothane anesthesia in O2/N2O (30%/70%). $MnCl_2$ (200nL 0.3M) was stereotactically injected into the somatosensory cortex S1, 1.5 mm below the dura, using a calibrated microcapillary. *MRI parameters:* DTI was acquired on a 11.7T BioSpec system (Bruker BioSpin, Ettlingen) with 750mT/m gradient sets, with 2D multi-slice diffusion tensor EPI with TR=3.9 s / TE=20.4 ms; in-plane resolution=156x156 μm^2; slice thickness=0.5 mm; 30 independent gradient directions; small/big-deltas=3/10ms; b-value=0 and 670 s/mm^2; nav=2; experiment time=33min. DTI maps and fractional anisotropy (FA) maps and fiber-tracking were calculated with DTI-studio software and ImageJ. MEMRI was performed 24h after injection, either at 11.7T with MP-RAGE sequence flip=10°; TR = 4.4 s / TE =4 ms; in-plane resolution= 98x98 μm^2; slice thickness = 0.5 mm; TI=1s; or at 7T with the same sequence flip=7°; TR = 4.4 s / TE =4.55 ms; in-plane resolution= 100x100 μm^2; slice thickness = 0.5 mm; TI=750ms. For fMRI studies of the electrical forepaw stimulation paradigm, coronal multislice spin-echo (SE) EPI images were acquired at 7T (with TE=30ms), or 11.7T (with TE=25ms) using the parameters described before[2]. BOLD activation maps were calculated with paired Student's t test ($p < 0.01$), using the software STIMULATE.

Results and discussion: During pre-stroke control conditions, both hemispheres are indistinguishable on DTI maps, MEMRI shows intact neuronal tracing between cortex and thalamus, and fMRI shows activation of the somatosensory cortices upon stimulation of the corresponding forepaw (Fig. 1). Following stroke, fMRI BOLD signal is lost on the ischemic cortex and diffusion anisotropy is decreased across a large part of the ischemic territory, as seen on DTI and FA maps. During the chronic observation period of 8 weeks, dynamical pathophysiological changes were followed. The lesion size increases over time, seen on T2 maps in agreement with extending area with loss of FA. DTI maps presented deterioration of fiber structures and also rearrangements of tissue. Even details such as transient compression of the hippocampus on the ischemic hemisphere is noted during the peak period of brain swelling due to the vasogenic edema (Fig. 2, red arrows). Upon resorption of edema, also diffusion anisotropy in the hippocampal structures is restored. When the corticothalamic connections were not affected by the insult, MEMRI showed manganese transport to the thalamus. However, in those cases when connectivity between the S1 somatosensory cortex and the thalamus was altered by the infarct we could not observed any manganese-induced hyperintensity in the thalamus, revealing no anterograde transport and confirming fMRI results.

Conclusions: The combination of these three MR imaging tools, MEMRI, DTI and fMRI provide important information on the interrelation between structural and functional deficits induced by stroke and the following evolution of pathophysiological events. Ongoing studies with higher temporal resolution on the various changes is expected to provide important prognostic clues about the structural correlate for the observed functional deficit following stroke, and even more importantly, about the chances for spotaneous recovery at often delayed periods.

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1. **References:**Weber, R., Ramos-Cabrer, P., Wiedermann, D., Van Camp, N., Hoehn, M. 2006. A fully noninvasive and robust experimental protocol for longitudinal fMRI studies in the rat. Neuroimage 29(4), 1303-1310.

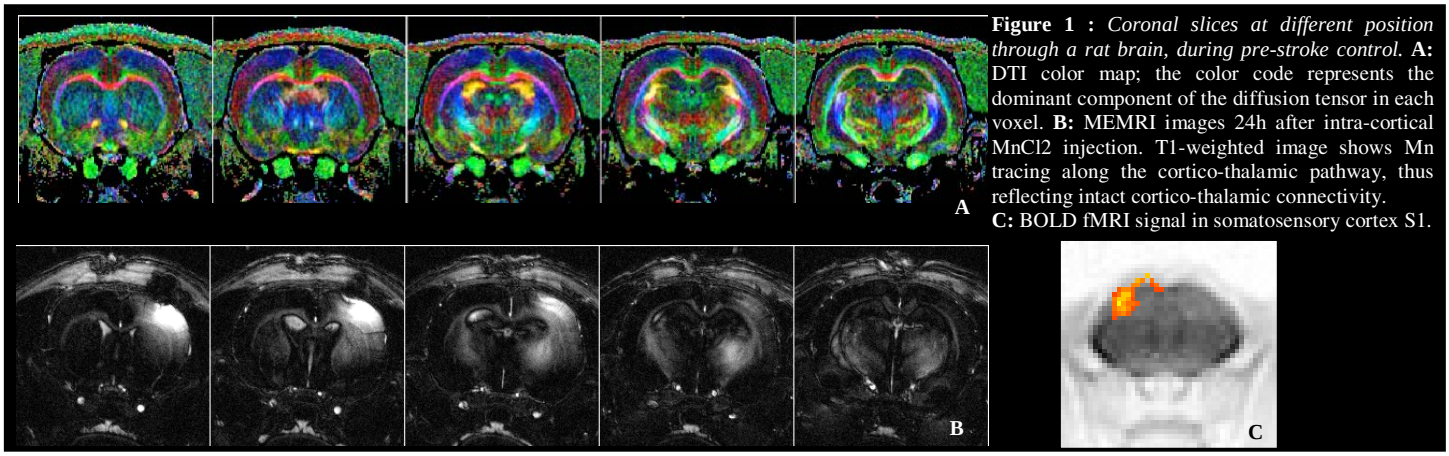

Figure 1 : *Coronal slices at different position through a rat brain, during pre-stroke control.* **A:** DTI color map; the color code represents the dominant component of the diffusion tensor in each voxel. **B:** MEMRI images 24h after intra-cortical MnCl2 injection. T1-weighted image shows Mn tracing along the cortico-thalamic pathway, thus reflecting intact cortico-thalamic connectivity. **C:** BOLD fMRI signal in somatosensory cortex S1.

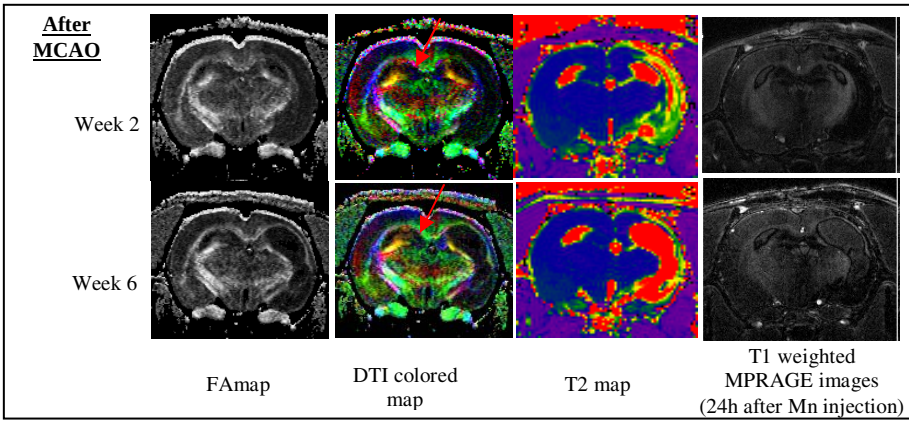

Figure 2 : Various coronal images of one rat at two post-stroke time points. FA map and T2 map demonstrate dynamics of lesion after stroke. DTI color map provides information about brain tissue orientational changes and fiber deterioration over time. MP-RAGE results after 24hr shows no Mn transport in the thalamus.