Mapping functional brain plasticity following spinal cord injury in rats: A combined fMRI and MEMRI study

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INTRODUCTION Over the past 30 to 40 years numerous research articles focused on elucidating the mechanisms of spinal cord injury (SCI) with the complex pathophysiologic processes slowly being unraveled. Based on anatomical studies there is evidence for reorganization in the rat sensory-motor cortex after spinal cord lesion [1]. In behavioral tasks animals showed spontaneous recovery and also functional compensation. Previous fMRI studies focusing on hind limb stimulation showed the absence of the BOLD signal after lesioning at the level of T8. However, it is yet not clear if the sensory map of the forelimbs changes in parallel to the physiologic motor map.

In this study we applied a sensory stimulation paradigm to a SCI model for a detailed analysis of the BOLD response and the number of voxels in the activated S1 forelimb area. In a complementary study, we used manganese enhanced MRI (MEMRI) to test if the hind limb area is still active and thereby generates output signals, even if there is no sensory input. Mn^{2+} accumulation and transport into the motor cortex output layer (white matter) reflects the neuronal activity integrated over the time interval between Mn^{2+} administration and MEMRI measurement and can thus be used as marker of the integrity of the trajectory [2].

METHODS Animal model: Male Lewis rats of 300g body weight have been used for the experiments. Rats underwent SCI (complete dorsal lesion) at T8. Following this intervention, the hind paws of the rats were paralyzed. MRI experiments were carried out at various time points following lesioning: Group 1 (n=6) prior as well as 4 and 8 weeks after lesioning, Group 2 (n=4) only one week after lesioning. For the MRI experiments, the animals were anaesthetized in an induction chamber, intubated and ventilated maintaining anesthesia with 1.1% isoflurane. A single dose of gallamine was given to reduce motion artifacts and also activation of the motor cortex. Blood CO_2 level and temperature was kept in a physiological range. All experiments were performed on a Biospec 9.4T horizontal-bore MR system (Bruker BioSpin AG, Karlsruhe, Germany) and were in strict adherence to the Swiss law for Animal Protection.

fMRI: For fMRI experiments a BOLD spin echo - echo planar imaging (SE-EPI) sequence has been used with the following parameters: echo time/repetition time (TE/TR): 10/1250ms, image matrix: 64x64, field of view (FOV): 3.3x2.5 cm^2, number of averages (NA): 8, temporal resolution: 10s, number of repetitions (NR): 50; two slices of 1.3mm thickness and interslice distance of 0.8mm have been recorded. The sensory stimulation paradigm consisted of sequential bilateral forepaw stimulation with subcutaneous electrodes following a block design with amplitude 6mA and stimulation frequency 3Hz. The on- and off-periods were 40s and 60s, respectively. This cycle was repeated 5 times.

MEMRI: Paramagnetic divalent Mn^{2+}, an MRI contrast agent, was injected stereotactically into the hind limb motor cortex area (M1). Mn^{2+} enters cells through voltage-gated calcium channels as a surrogate for Ca^{2+}[3]. Both efficient Mn^{2+} uptake into neurons and its transsynaptic transport are dependent on neuronal activity. Tissue uptake of Mn^{2+} was analyzed on the basis of changes in signal intensity in T_1-weighted images recorded 10h after stereotactic injection of a 20µl solution containing 800mM of $MnCl_2$ into the motor hind limb area. Three groups of animals were measured: non lesioned (n=4), one week (n=4) and 4 weeks (n=4) after lesioning. A T_1-weighted 3D RARE sequence was used with the following parameters: TE/TR: 7.9/200ms, image matrix: 256x128x64, FOV: 2.8x2.6x2.8mm^3, NA: 2.

Data analysis: Data analysis was carried out using Biomap (4th version, M.Rausch, Novartis, Basel, Switzerland). For statistical analysis of the effect of peripheral stimulation on brain activity, parametric maps were calculated by using the general linear model (GLM). For calculation of the statistical maps a threshold p=0.05 was applied. In addition, activation clusters had to be larger than 5 voxels. For analysis of MEMRI data, focus was set on the M1 output layer. Regions of interest were drawn over several slices. Signal intensity and volume were normalized to muscle.

RESULTS fMRI: No difference was found between left and right forepaw stimulation. Consequently, fMRI data from both sides were pooled. A comparison of the BOLD signal change over time (in percent of the baseline intensity, ΔBOLD) revealed a decrease in the post-lesion compared to the pre-lesion animals. This effect was most obvious one week after lesion (Fig. 1a). For statistical comparison the BOLD signal change was integrated over time. Animals prior to SCI showed significantly larger ΔBOLD values than all groups following lesioning (one way ANOVA, Fig.2a). One week after lesioning the number of voxels (volume) displaying activity tended to be larger than the number prior to SCI (one way ANOVA, p=0.06, Fig.2b).

MEMRI: There was a trend for reduced manganese uptake in the M1 output layer one week and four weeks post lesion reflecting less activity in hind limb area projection neurons.

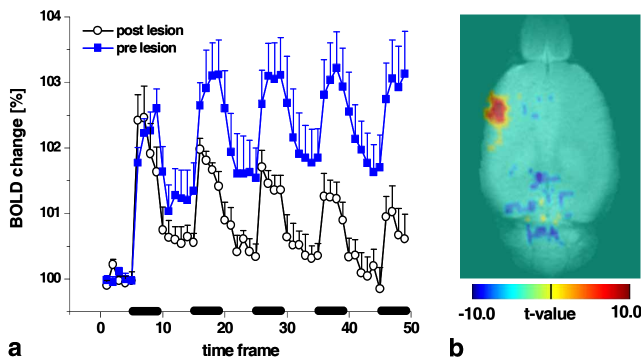

Figure 1: (a) Relative change of the BOLD signal during electrical forepaw stimulation (black bars) before (6 animals) and one week after SC lesion (4 animals). **(b)** Activation map showing right forepaw response of one animal one week after SC lesion.

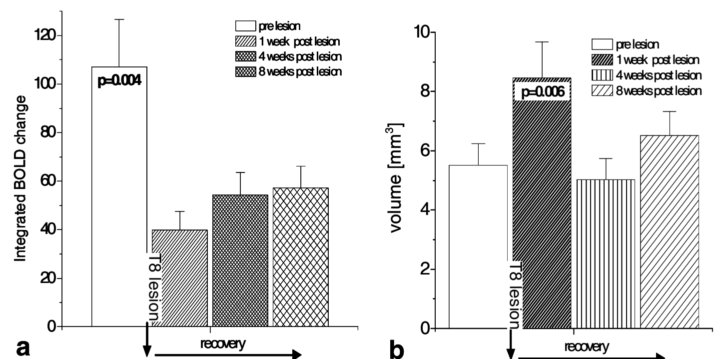

Figure 2: Statistical comparison (ANOVA, p-values are indicated) of the BOLD signal integrated over time **(a)** and of the size of the activation area **(b)** before lesion and 1, 4, and 8 weeks after lesion.

CONCLUSIONS Our studies indicate an involvement of the forelimb area in cortical reorganization in this model of SCI. Furthermore, this work shows the potential of fMRI for monitoring brain plasticity in rodent models being an attractive complementary *in vivo* readout for longitudinal studies and potential therapeutic pre-clinical trials.

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