Longitudinal fMRI in rats following spinal cord lesion and spontaneous functional improvement

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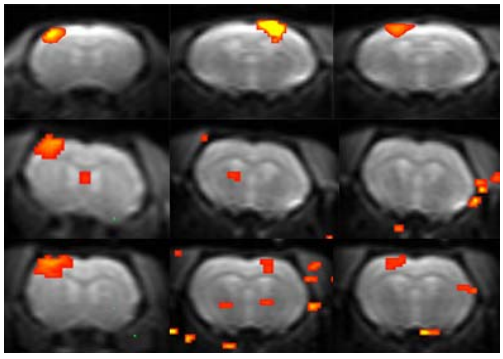
Background: After spinal cord injury (SCI), a scar forms within a week which has been shown in the rat SCI model(1) to impede axonal regeneration by forming a physical and chemical barrier for regenerating axons. But previous studies also reported spontaneous regeneration of motor functions assessed with behavioral tests after partial transection of the cortico-spinal tract (CST). To be able to differentiate between spontaneous regeneration, plastic cerebral reorganization or therapeutical effects as causes for the behavioral improvements, information about the preservation of the afferent system, parallel to the efferent system, is helpful. Here, we present an investigation on the recovery of the somatosensory system in female rats after SCI using longitudinal fMRI studies. The main challenge of such longitudinal fMRI is a suitable anesthesia. Starting from our medetomidine anesthesia protocol for male Wistar rats(2), initial experiments showed that female rats of the same strain need a different dosage of the anesthesic.
In the present study we have

- established a medetomidine anesthesia for female Wistar rats suitable for robust and repetitive fMRI
- defined a measurement protocol to perform reliable, longitudinal fMRI in spinal cord lesion model
- followed up spinal cord lesions with fMRI in the rat model up to 11 weeks post injury

Methods:
Animal model: In 4 adult female Wistar rats after laminectomy the dorsal CST was transected with a Scouten wire knife at thoracic level 8. After transection the dura was sutured. This lesion affects motor functions of the hindlimbs but not the forelimbs. Additionally, bladder emptying is disturbed in the first week during the acute phase due to the spinal shock and may lead to adverse effects. Female rats were used because they allow the manual emptying of the bladder which was done regularly during the first week.
fMRI Anesthesia: Medetomidine (Domitor): After a short, initial isoflurane anesthesia, medetomidine anesthesia was started with a bolus of 0.035 mg/kg bodyweight followed by an s.c. infusion of 0.1 mg/kg bodyweight per hour.
MR-imaging was performed on a 7T BioSpec animal scanner (Bruker BioSpin, Ettlingen, Germany), equipped with home-built rf-coils; we used a 10 cm Helmholtz coil for transmission and a 2 cm diameter surface coil for signal detection. Our imaging protocol consisted of localizer scans for animal positioning, anatomical imaging and SE-EPI BOLD experiments, TE/TR=30/3000ms; FOV=2.56 cm; matrix 64^2. Imaging was performed 1, 3, 5, 7, 9, and 11 weeks after spinal cord injury; after the last scan the animals were sacrificed and perfusion fixated.

fMRI setup: Electrical paw stimulation was perfomed with 2mA rectangular pulses at a frequency of 3 Hz. We used a block paradigm with stimulation duration of 15s followed by a 45s off period, repeated 5 times. After each fMRI scan a 10 min rest / silence period was preserved. Functional imaging was started with stimulation of an unaffected forepaw to guarantee a robust BOLD fMRI signal. After confirmed BOLD with the healthy forepaw, fMRI was performed twice for each impaired hindpaw. As further control at the end of each session, fMRI of healthy forepaw stimulation was acquired again to confirm persistent normal physiological condition necessary for observation of the BOLD effect.
Histology: The spinal cord was cryo sectioned in 50µm thick parasagittal slices. The collagenous scar was detected by applying trichrome staining.

Results:
The anesthesia was successfully modified for female rats and proves to be stable and reliable. The initial bolus had to be lowered compared to male Wistar rats (0.035 vs. 0.05 mg/kg BW) whereas the continuous infusion rate could be kept constant. BOLD signal could be observed in every animal when stimulating a non-affected forepaw. In single occasions physiological conditions changed during the session, indicated by the weakened or even absent BOLD contrast in the last scan of the healthy forepaw. In general, the animals showed a consistent BOLD signal with the healthy forepaw throughout the study but there is also markedly inter-individual variation in significance and activation size for the forepaw stimulation, which correlates with the severity of the lesion. Additionally, in all animals reoccurring BOLD contrast of small amplitude could be observed with hindpaw stimulation, in one animal after 3, two after 5 and one after 7 weeks post lesion. Animals with smaller lesions and without cyst formation in the lesion were recovering within the first 3 weeks whereas animals with more severe lesions were showing a BOLD contrast only at later timepoints (5 or 7 weeks).



Conclusions: We have successfully adapted the experimental protocol to work for female Wistar rats, and have defined an fMRI protocol to reliably detect small BOLD contrast. It was possible to follow up the individual reoccurrence of BOLD contrast after experimental spinal cord lesion with longitudinal fMRI. The results indicate that longitudinal fMRI in a spinal cord lesion model is feasible with good sensitivity and reliability. We will use this technique to study the beneficial effect of an anti-scarring treatment after spinal cord injury.

Fig. 1: Longitudinal fMRI in 3 rats: from left to right: stimulation of healthy right forepaw / left hindpaw / right hindpaw. Top: healthy control; middle row: SCI rat without reoccurring BOLD; bottom row: SCI rat with bilateral BOLD stimulating the impaired hindpaws after 3 weeks post lesion.

References: 1) Hermanns S., Klapka N., Mueller HW. Restor Neurol Neurosci **19**, 139-148 (2001)
2) Weber R, Ramos-Cabrer P, Wiedermann D, van Camp N, Hoehn M. Neuroimage. 29, 1303-10 (2006).