Somatotopic plasticity of the BOLD response following spinal cord injury in rats: use of anatomical landmarks on EPI images for accurate topological mapping

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Introduction The accuracy, at which changes in cortical functional topology can be assessed with fMRI methods, depends on the quality of the reference coordinate system that is used for comparison of data sets obtained in different imaging sessions[2][3]. In this study we monitored plastic changes in the somatosensory areas in rats following spinal cord injury (SCI) over a time period of 12 weeks using blood oxygen level depended (BOLD) fMRI methods in combination with electrical stimulation of fore and hind paws. Mapping of the imaging data on the reference coordinate system is based on a highly reproducible artifact caused by the Bregma line on coronal sections in echo planar (EPI) images recorded at 9.4T. This landmark can be used to map and elastically scale EPI images of individual animals to the Paxinos[1] atlas coordinate system thereby circumventing potential errors which inevitably appear if activation clusters are overlaid on high-resolution anatomic images devoid of susceptibility artifacts and distortion associated with EPI images. The accuracy of the image registration procedure was evaluated by analyzing the sensory representation of fore- and hindpaw in terms of reproducibility within and across rats and by comparison with fMRI and electrophysiological literature data. In a second step the method was applied to investigate plastic events in the rat sensory cortex following SCI. Changes in the functional response to sensory stimulation were not only analyzed regarding changes in the amplitude of the BOLD signal or area of the activated region but also regarding alteration in border zone between the fore and hind paw somatosensory area best detectable on coronal sections.

Methods : Animal model: The SCI, an incomplete hemisection at level C3/C4, was performed in Lewis rats at post-natal day 28. The injury let the dorsal funiculus and corticospinal tract intact, only affecting the spinothalamic and the spinocervical tracts. Twelve weeks later 9 injured animals and 7 age-matched control animals were studied using fMRI. As there was significant variation in the severity of the lesion post-mortem reconstructions of the injury site was used to select animals for the study. Finally four animals with similar extent of the injury were included for this analysis.

For the fMRI experiments, the animals were anaesthetized, intubated and artificially ventilated maintaining anesthesia with 1.5% isoflurane. A single dose of gallamine was administered to reduce motion artifacts. Blood CO_2 level and temperature were kept withinin a physiological range. All experiments were carried out in strict adherence to the Swiss law for Animal Protection.

fMRI: fMRI experiments were performed on a Biospec 9.4T horizontal-bore MR system (Bruker BioSpin AG, Karlsruhe, Germany). A gradient echo – echo planar imaging (GE-EPI) sequence has been used with the following parameters: echo time/repetition time (TE/TR): 10/1250ms, image matrix: 64x64, field of view (FOV): 3.3x2.5cm^2, number of averages (NA): 8, temporal resolution: 10s number of repetitions (NR): 50; two slices of 1.3mmmm thickness and interslice distance of 0.8mmmm have been recorded. The sensory stimulation paradigm consisted of sequential bilateral forepaw stimulation with subcutaneous electrodes following a block design with amplitude 6 mA and stimulation frequency of 3 Hz. The on- and off periods were 40s and 60s, respectively. The on/off cycle was repeated 5 times.

Data analysis: Data analysis was carried out using Biomap (4th version, M. Rausch, Novartis Institute for Biomedical Research, 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 calculation of the somatotopic coordinates an in-house developed IDL based software has been used.

Results: Fig.1 A) shows a representative EPI coronal section with an overlay of the activated area in S1 following forepaw stimulation. The mark indicates a reference point of the coordinate system. The scheme inset in Fig. 1A) illustrates the rat spinal cord with the scaffold area representing the extent of the injury. Fig.1B) shows the BOLD response as a function of time for control and SCI rats. For control animals the signals from the paw ipsi- and contralateral to the injury were averaged. For injured animals the responses for both paws (ipsi and contralateral to the lesion) are shown. Strikingly both paws show the same response dynamics and clearly differ from control animals. Fig. 2A) shows the somatosensory topology of control rats for fore- and hindpaw area. Fig.2B) clearly illustrates how the responsive area in injured animals expands over the former forepaw area (drawn as outline), with a significant (p<0.01) increase of the activated forepaw area in the SCI rats, Fig.2C).

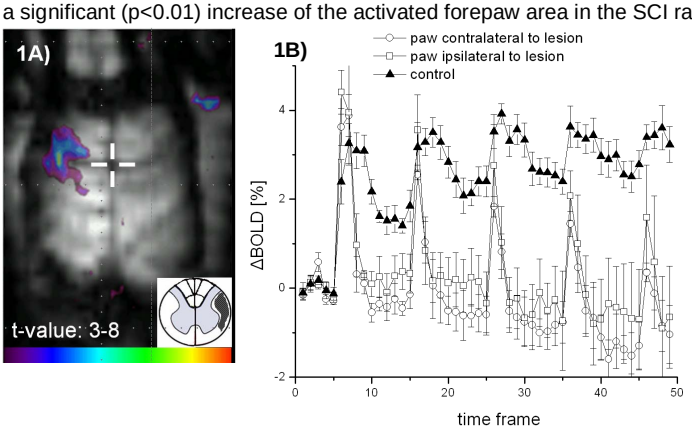

Figure1: A) Coronal EPI image with activation map showing landmarks for the reference system. B) Relative change of the BOLD signal during electrical forepaw stimulation for control animals and injured animals (paw ipsi and contralateral to lesion site are shown). The inlet in A) shows a scheme of the injury (scaffold area).

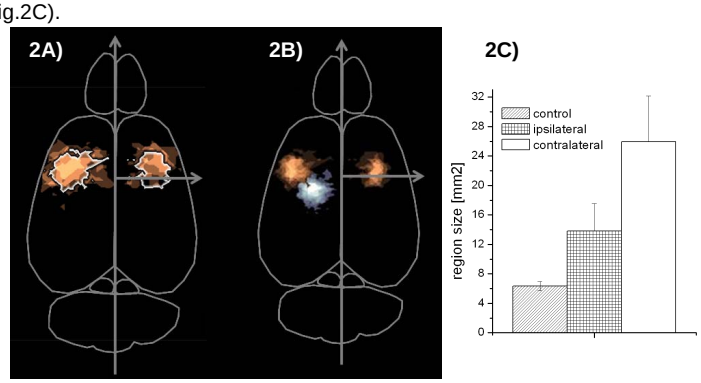

Figure2: A) Maps after injury, former forepaw maps are drawn as outline. B) Somatotopic mapping of left and right forepaw and right hindpaw. C) Statistical comparison of the size of the activation area (ANOVA, p<0.01)

Conclusions: 1) The studies in control animals demonstrate the high accuracy of the method for somatotopic mapping. 2) Differences in the dynamic pattern indicate the absence of "slow components" in the fMRI response of SCI animals. This may be tentatively attributed to a contribution of the spinothalamic tracts, which consist of nociceptive-specific and wide-dynamic range neurons and project to the contralateral side of the spinal cord. 3) Spatial enlarging of the responsive area indicates plastic changes in somatosensory forepaw region of SCI rats. 4) Detailed analysis of plastic events critically depend on the availability of accurate registration tools.

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