FUNCTIONAL MAGNETIC RESONANCE IMAGING REVEALS BRAIN CORTEX REMODELING IN A RAT MODEL OF CHRONIC MULTIPLE SCLEROSIS

Stefano Tambalo[1], Silvia Fiorini[1], Roberta Rigolio[2], Pietro Bontempi[1], Andrea Sbarbati[3], Guido Cavaletti[2], Paola Marmiroli[2], Stefano Pluchino[4], and Pasquina Marzola[5]

[1]Dept. of Neuological and Movement Science, Università di Verona, Verona, Verona, Italy, [2]Dept. of Surgery and Translational Medicine, Università Milano-Bicocca, Milano, Italy, [3]Dept. of Neuological and Movement Science, INSTM Firenze, Verona, Italy, [4]Dept of Clinical Neurosciences, John Van Geest Centre for Brain Repair, Stem Cell Institute and NIHR, University of Cambridge, Cambridge, United Kingdom, [5]Dept. of Informatics, University of Verona, Verona, Italy

Purpose Multiple Sclerosis (MS) is a chronic demyelinating disease of the central nervous system. During the last years functional Magnetic Resonance Imaging (fMRI) studies have shown that cortical reorganization consistently occurs both in acute and clinically stable MS patients. Experimental Autoimmune Encephalomyelitis (EAE) is considered a good model of MS both in rodents and non-human primates. In the present work, we have applied fMRI with somatosensory stimulation and invesigated the alteration in functional response in rats with induced chronic EAE. We demonstrate that the evoked cortical activation is strongly altered in EAE rat brain compared to controls. In addition, we performed Diffusion Tensor Imaging (DTI) experiments to assess and evaluate the evolution of white matter lesions in brains of rats affected by EAE.

Methods EAE was induced in male DA rats (n=15) by hindlimb footpad injection of 100mg syngenic spinal cord homogenate. Evaluation of clinical score was performed from 9 up to 60-64 days post injection (dpi). The score was assessed in a scale ranging from 0 to 5 where 0: healthy animals and 5: moribund or dead. Prior to EAE induction, 8 on 15 randomly chosen rats, were investigated by fMRI. After EAE induction, fMRI was performed 30 and 60 dpi. To increase the sensitivity of the experiment, a SPIO contrast agent was administered. Electrical stimulation was delivered through needle electrodes inserted in the right forepaw with a square pulse wave with frequency=7Hz, current=2mA, duration=0.5 ms. The block design paradigm consisted of a rest period (60 s) followed by a stimulation period (20 s); this paradigm was repeated 6 times for a total of 240 EPI volumes acquired with a temporal resolution of 2s. The stimulation train was acquired three to four times on each subject with a time interval of about 10 minutes between scans. An EPI diffusion weighted sequence was also employed to acquire images at the three time points: 20 axial slices covering the whole brain, 30 diffusion directions and b=1000 s/mm^2.

Results The neurological score of rats followed a time trend with the peak of EAE around 14 dpi followed by remission, while the animals experimented disease relapse at different time points between 20 and 40 dpi. In healthy DA rats, fMRI revealed that most of the activated volume was observed in the controlateral hemisphere and specifically in the S1 cortex with a relevant percentage in the motor cortex. On 30 dpi, the activation pattern was substantially altered compared to the pre-induction stage and heterogeneous among different EAE rats. Activation was also detected in the ipsilateral right cortex and in some extra-cortical areas. On 60 dpi the activation maps were acquired in rats that had been already studied on 30 dpi, so it was possible to investigate the activation process along with the disease course. The qualitative comparison of fMRI maps (Fig. 1) showed an increased activated volume after EAE induction in all investigated rats at both time points, despite better clinical conditions recorded at 60 dpi. The total activated volume increased by about 80% ($p<0.01$) on 60 dpi vs. 30 dpi (data not shown). The number of activated pixels did not correlate with clinical score achieved by each animal. The Laterality Index (LI) is an index of hemispheric dominance in functional response. In our MS model, LI strongly decreased on 30 dpi (0.02± 0.16) compared to the pre-induction value (0.97±0.09), while it significantly increased ($p<0.05$) on 60 dpi (0.12 ± 0.19), thus remaining definitely lower than LI in healthy animals. We performed a deeper analysis to clarify functional remodeling. Before EAE induction, the activated volume was mainly restricted to the cortex S1 and in the motor cortex of the left hemisphere. At 30 dpi, most of the activated volume was still observable in S1 and motor cortex with similar values for the left and right hemispheres. Several other brain regions were substantially involved in the functional response with statistically significant difference between left and right emipshere in some regions. Similar findings were observed at 60 dpi, but an inter-animal reduction of variability suggests a more homogeneous activation pattern in advanced stages of EAE. We also considered the effects of EAE on the percentage of activated voxel in specific brain regions: a generalized increase was observed in all EAE animals, reflecting the strong increase in the total activation volume. DTI experiments showed significative decrease ($p<0.05$) in Fractional Anisotropy (CTRL 0.31±0,02, 30dpi 0.26±0,02, 60dpi 0.27±0,02) and Axial Diffusivity (CTRL 0.0013±0,0001 mm^2/s, 30dpi 0.0010±0,0001 mm^2/s and 60dpi 0.0011±0,0001 mm^2/s) when evaluating the whole brain.

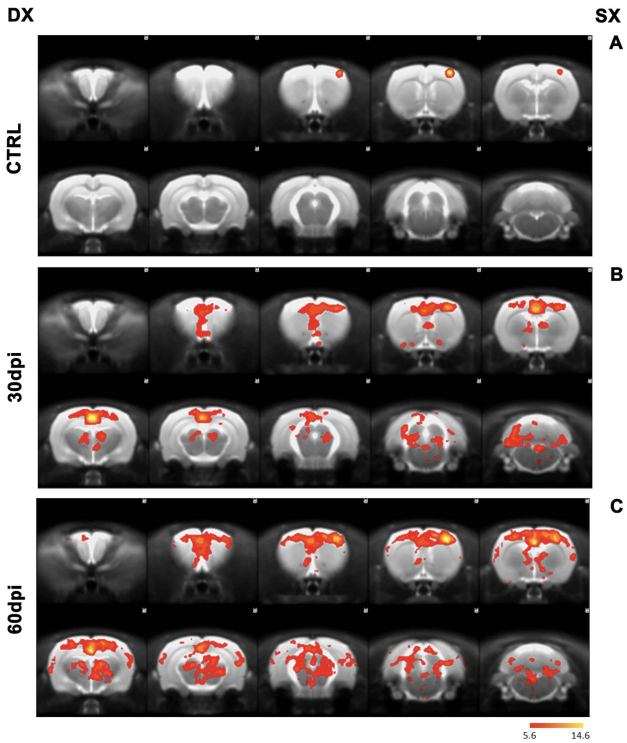

Figure 1: Functional activation maps in EAE rats before (A), 30 (B) and 60 (C) days post induction.

Conclusion The present work is the first attempt to define a reliable model to dissect the brain plasticity phenomenon occurring in MS patients. Moreover such a tool could be also used as a sensitive indicator of therapeutic intervention in preclinical studies on the brain and/or spinal cord of the affected animals. This work was supported by Fondazione Italiana Sclerosi Multipla (FISM) for grant support (FISM 10/12/F14 /2011).

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