EXPLORING THE MOUSE BRAIN FUNCTIONAL NETWORKS AFTER DEMYELINATION: A RS-FMRI STUDY

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Introduction

In Multiple Sclerosis (MS), resting state functional magnetic resonance imaging (rs-fMRI) is a valuable tool to improve the knowledge about the pathophysiology of functional impairments observed in patients and it is widely used to evaluate the alterations in brain functional connectivity (FC) [1]. However, for analysis of the mechanisms underlying the development of myelin disorders as well as for the development of new therapeutic strategies, translational studies in mouse models of brain disorders are of great importance. The cuprizone mouse model is a well established model for myelin pathology, with a chronic state of demyelination after twelve weeks of cuprizone treatment [2]. **The main goal** of our study was to investigate with rs-fMRI the impact of experimentally induced demyelination on the functional connectivity networks of cuprizone demyelinated mice. Large-scale neuronal networks were probed in severe demyelinated and control animals, performing rsfMRI at 7 Tesla and employing a mouse head adapted cryoprobe. Independent component analysis (ICA) and ICASSO [3] combined with partial correlation analysis and graph theory [4] were used to look for pathologically induced FC alterations or for identifying remodeling features resulting from possible compensatory mechanisms.

Material and Methods

Female 8-week old C57BL/6 mice were fed with 0.2% cuprizone for 12 weeks (n=8) to induce chronic demyelination. A group of 8-week old C57BL/6 mice was kept as control (n=7). Rs-fMRI examinations were performed at the end of cuprizone treatment. Duplicates for each group were kept in the same conditions of housing and treatment and were used for the histopathological examination. Data Acquisition: Mice were scanned under moderate medetomidine sedation using a 7 T small bore animal scanner scanner (Biospec 70/20, Bruker, Germany) and a mouse head adapted cryocoil (MRI CryoProbe, Bruker, Germany). Images were acquired at constant levels of blood oxygen saturation and body temperature. Rs-fMRI was performed using a single shot Gradient Echo EPI (TE/TR = 10ms/1700ms). The whole mouse brain (excluding the cerebellum) was covered using 12 slices with a slice thickness of 0.7 mm, a field of view of 192 x 12 mm² and an acquisition matrix of 128 x 80. The obtained image resolution was 0.15x0.15x0.7 mm^3. 200 volumes were recorded in interlaced fashion for each run. Data Analysis: Data were preprocessed using the MATLAB Toolbox for statistical parametric mapping (SPM8). Realignment of the 200 volumes to the first one was done to correct for motion. Data was spatially normalized and aligned to a template. Smoothing was performed with a full width at half-maximum Gaussian kernel of 0.4x0.4x1 mm³. Group Independent Component Analysis (ICA) using the MATLAB tool GIFT (Group ICA of fMRI Toolbox, v1.3i, Calhoun et al.) was performed with the datasets of all animals, asking for 100 components. Bootstrapping ICASSO runs with 20 repetitions were used to check for algorithmic stability and reproducibility. Time courses of the obtained 100 ICA components were further used for partial correlation (PC) analysis, separately performed for the demyelinated and control groups. This resulted into two PC matrices, averaged across the demyelinated and the control group, respectively. These graphs represented the ICA components (brain region) as nodes, and assigned the degree of correlation in their response profile (weight). From this, the mean strength for each node was calculated. We further generated binary matrices with positive correlations only, using a threshold of $p<0.05$ for assessing the number of statistically relevant connections. Graph theory including fine tuning was used for calculating the modularity to find functional connectivity networks.

Results and Discussion

Demyelination was induced after twelve weeks of cuprizone treatment. T2-weighted images of the mouse brain in figure 1 show myelin loss in the corpus callosum and external capsule of cuprizone treated mice compared with controls. 100 component group ICA resulted in bilateral and unilateral patterns of activation which could be assigned to anatomically well-defined brain regions, down to distinct, individually detected brain nuclei. Examples of biologically relevant components are shown in figure 2A with left retrosplenial agranular cortex - RSA (I), left hippocampus (II), left visual cortex - V1/V2 (III) and left barrel cortex - S1BF (IV). ICA with bootstrapping ICASSO runs with 20 repetitions resulted in a clustering index I_q >0.8 in 71% of the 100 components, indicating a high stability and reproducibility of the resulting activation patterns. Partial correlation and graph theory analysis partitioned the FC networks (modules) for each group respectively. Interestingly, an supplementary functional module was obtained in the cuprizone treated group when compared with controls, indicating a higher degree of functional segregation. Applying fine tuning [6] the number of functional connectivity networks was not changing and the modules remained stable in controls whereas modifications were identified for the allocation of single components in the cuprizone treated group. The additional module identified for the cuprizone animals is grouping cortical areas of motor and sensory systems but also regions belonging to the limbic system (hippocampus and amygdala). Moreover, a reorganisation of the cortical areas in modules was observed in the cuprizone treated group. As measures of centrality, number of relevant connections and the mean strength were also assessed for each group separately, in order to identify the relevant, influential nodes, considered to be critical for coordinating functional interactions. The average number of relevant connections for a component is much higher in the group of cuprizone treated animals (mean = 13) compared to controls (mean = 9), suggesting a remodeling of the networks towards increasing the number of interacting areas. The mean strength of the nodes is however decreased in the cuprizone grup. In figure 2B (controls) and 2C (cuprizone) the components with the highest mean strengths are displayed. 78% of these components are the same in both groups but differing in ranking. Components present only in one group are shown in red.

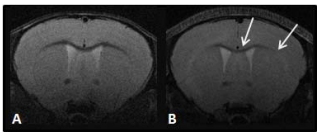

Figure 1: White matter loss in corpus callosum during Cuprizone treatment visible in T2-weighted MRI images of the mouse brain (A) control animals (B) Cuprizone treated animals

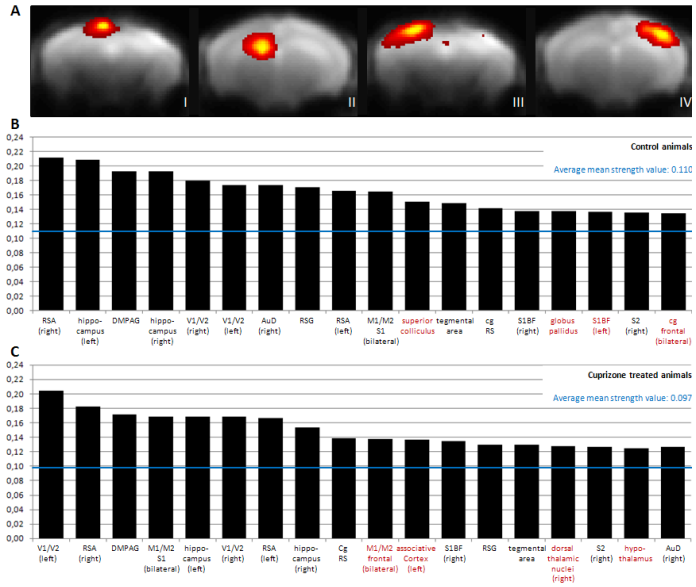

Figure 2: (A) Examples of activation pattern obtained by group ICA (I) retrosplenial agranular cortex, RSA (II) hippocampus (III) visual cortex, V1/V2 (IV) barrel cortex, S1BF (B,C) components with the highest mean strength values obtained by partial correlation analysis in control animals (B) and cuprizone treated animals (C); abbreviations adapted from Paxinos mouse brain atlas [5]

Conclusion: This study demonstrates the potential of rsfMRI in investigating the mouse brain functional networks in normal and pathological conditions. Here, we show preliminary data indicating a complex remodeling of the large scale functional networks in the mouse brain, as an effect of demyelinating pathology. Further analysis of our data will clarify aspects related to the precise identification of dysfunctional networks and the compensatory response.

References

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