The differential progression of functional and structural connectivity in a mouse model of demyelination and remyelination

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INTRODUCTION: Resting state fMRI has become a tool to study brain connectivity changes in development and disease. Although it is dependent on structural connectivity, functional connectivity can be maintained by remnants of structural connectivity, even indirectly [1, 2]. Thus when structural connectivity is altered, the question arises as to how the relationship between functional and structural connectivity changes with time, which has implications for the interpretation of clinical findings. Using the cuprizone mouse model of reversible demyelination [3], we tracked the progression of demyelination in mice and their subsequent remyelination, and compared the temporal changes of functional connectivity to that of corpus callosum myelination using histology.

METHODS: **Experimental design**: 8-week-old C57BL/6 male mice (n=3) were fed 0.2% (w/w) cuprizone mixed in milled chow for 6 weeks, then switched to normal chew for another 6 weeks. They were imaged at three time points (3rd , 6th and 12th week from start of cuprizone diet), on a 9.4T scanner (Agilent Technologies, USA), under a protocol optimized for mouse resting-state fMRI (0.6mg/kg/h medetomidine i.p. [4]). fMRI of both resting state and forepaw stimulation (6Hz, 0.5mA) was performed. A 2nd group of healthy C57BL/6 mice (n=3) were scanned with the same fMRI protocol as controls. A 3rd group of C57BL/6 mice (n=9) went through the same cuprizone regimen and were sacrificed at 9 different time points to assess myelination using Luxol fast blue staining. **MRI**: BOLD EPI: TR/TE=2000/15ms, α=90°, slices=15, voxel=0.31x0.31x0.5mm^3, volumes (resting state / forepaw stimulation) = 300/150. FSE: TR/TE=2500/40ms, echo-train=8, average=2, voxel=0.08x0.08x0.15mm^3. **Analyses**: Resting state fMRI: Data were bandpass-filtered at 0.01 and 0.1Hz. Spatial smoothing was done with Gaussian kernel of 1 pixel FHWM. Ventricle signals were regressed out to reduce physiological noise. Connectivity maps were generated by correlation (r>0.25, cluster threshold=4 voxels) using seed points, which were based on the S1 areas identified with forepaw stimulation. Correlations between time courses from bilateral ROIs of 4x4 voxels surrounding the seed points were analyzed within the three frequency bands of 0.01-0.04Hz, 0.04-0.07Hz and 0.07-0.1Hz. Statistical analyses were done using ANOVAs with post hoc tests (p-value threshold=0.05). Temporal SNR was calculated to assess the quality of the EPI scans across time.

RESULTS: The histology and functional connectivity results showed different temporal progression in response to the cuprizone diet (Figs. 1, 2). Bilateral S1 functional connectivity was already largely impaired at the 3rd week of the diet, even though the corpus callosum only showed mild demyelination then. Here, functional connectivity was particularly impaired in the frequency band from 0.01-0.04Hz (Fig. 3). However, when demyelination was most severe at the 6th week (the end of the diet), bilateral S1 regions showed strong synchrony especially in the very low frequency band, suggesting a slower oscillation. Moderate remyelination of the corpus callosum in week 12 (6 weeks after cessation of diet) correlate with maintenance of inter-hemispheric functional connectivity, even though the strength was slightly reduced (Fig. 2, 3). Fig. 4 shows that the correlations were not related to scan quality across sessions.

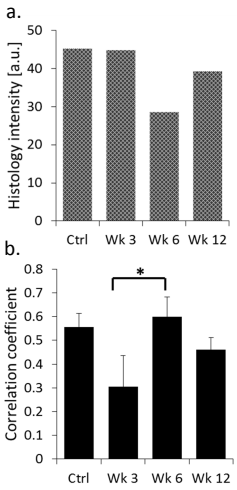

Fig. 2: (a) Corpus callosum histopathology from individual mice showed marked demyelination in week 6. (b) Correlations of bilateral S1 regions showed significant impairment at week 3, followed by strong regaining of bilateral connectivity at week 6. (Error bars: S.E.M.)

DISCUSSION: Targeted injury to structural connectivity, such as corpus callosum section [1] or demyelination (as in this study), causes the impairment of inter-hemispheric functional connectivity. Furthermore, we show that the trend of functional connectivity does not necessarily follow the trend of structural injury and recovery. In the cuprizone model of demyelination, which causes the death of oligodendrocytes largely around the corpus callosum and superior cerebellar peduncle [3], functional connectivity appears to be quickly affected, compared to the slower rate of demyelination observed. The strong rebound of functional connectivity at 6 weeks, even when the corpus callosum was most demyelinated, is reminiscent of the increases in functional connectivity seen in multiple sclerosis patients with white matter damage [5, 2]. In the latter study, stronger functional connectivity also correlated with worse cognitive scores, which raises the question of the physiological significance of increased functional connectivity. On the other hand, a decrease in functional connectivity, as found in other studies of multiple sclerosis patients, is usually associated with disease severity [6]. These contrasting results could be due to structural connectivity varying with disease progression, as also suggested by the trends seen in this preliminary study. Factoring in our observations of a return to moderate connectivity strength after remyelination, it appears that either end of the spectrum of functional connectivity strength can reflect underlying structural dysfunction.

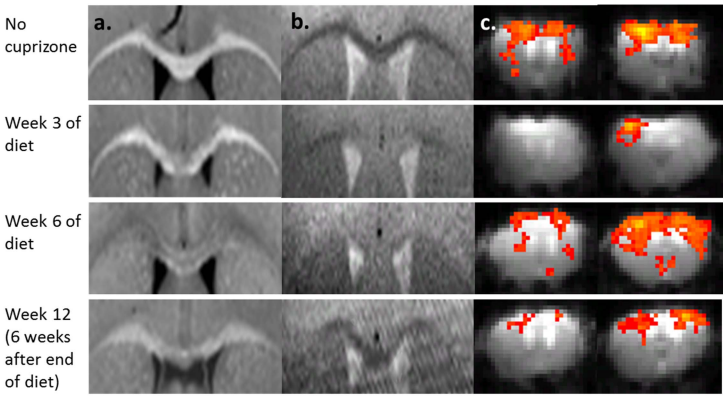

Fig. 1: Changes induced by the 6-week cuprizone diet across time. (a) Histology showed demyelination in the corpus callosum during the diet and remyelination at the 12th week (6 weeks after stopping the diet). (b) T2-weighted anatomical images showed similar demyelination and remyelination. (c) S1 functional connectivity maps (same mice in (b)) showed clear impairment at week 3, with strongly regained connectivity at week 6, and which was moderately maintained at week 12.

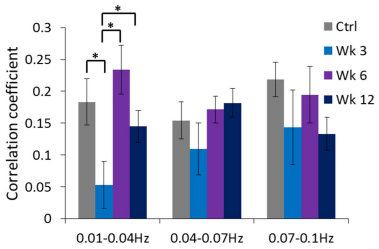

Fig. 3: Correlation broken down by three frequency bands: Bilateral S1 time courses in week 3 had significantly reduced synchrony in the lowest band of 0.01-0.04Hz, while they became highly synchronised in the same band in week 6 and returned to near normal levels in week 12. (Error bars: S.E.M.)

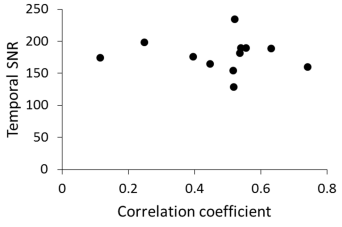

Fig. 4: Correlation coefficients between bilateral S1 regions did not relate to temporal SNR.

REFERENCES: [1] O'Reilly, 2013, PNAS 110:34; [2] Hawallek, 2011, PNAS 108:47; [3] Torkildsen, 2008, Acta Neurol Scand Supp 188:72; [4] Nasrallah, 2013 Neuroim (Epub); [5] Valsasina, 2011 Eur J Neurosci 33(7):1256; [6] Bonavita, 2011, Mult Scler 17(4):411.