MOTOR NETWORK EFFICIENCY AND DISABILITY IN MULTIPLE SCLEROSIS

SUPPLEMENTARY ONLINE MATERIALS:

SUPPLEMENTARY METHODS

Network based analysis: Identification of the motor network components

a) *GM mask generation*: Based on published models of motor function[14] masks of relevant GM regions were prepared in Montreal Neurological Institute (MNI) space (Figure 2) using the Harvard-Oxford cortical and subcortical structural atlas, included in FSL[12]. Left- and right-side masks were created separately for each region. Superior and middle cerebellar peduncle masks were also prepared as waypoints for cortico-cerebellar fibre tractography.

b) *Generation of Track Density Imaging (TDI) maps:* TDI is a post-processing method of diffusion images that takes into account the distribution of fibre tracts over the whole brain to better characterize tract trajectories at a local level, and has been shown to improve the extraction of tracts with complex trajectories.[S1,S2] TDI maps, representing the number of tracts passing within each element of a 1 mm^3 grid were computed using Constrained Spherical Deconvolution (CSD) tractography[15] as previously described.[S2]

c) *Registration of the GM masks from MNI space to each control native diffusion space*: GM masks were registered to each subject's TDI maps in native diffusion space using a non-linear transformation[S3] as previously described.[S4] Briefly, the MNI-152 template was registered non-linearly to a given subject's T1-weighted image; the subject's T1-weighted scan was registered to the High Angular Resolution Diffusion Imaging (HARDI) data via a pseudo-T1 image generated from the Proton Density/T2-weighted scan. The T1-weighted scan was rigidly registered to the pseudo-T1 image. The T2-weighted scan, used for creating the pseudo-T1 image, was non-linearly registered to the diffusion b=0 average image, which is in the same space as the TDI map. The transformations parameters were then combined to move the GM ROIs in MNI space to each subject's TDI map. GM masks in TDI space were then dilated to reach the neighbouring WM, so they could be more reliably used as seed points for WM tractography. The positioning of each mask was then checked by MP.

d) *Extraction of WM tracts connecting GM regions:* Tractography was performed on the eddy-current-corrected and brain extracted HARDI data in MRtrix[15] (www.brain.org.au/software/mrtrix/) using probabilistic CSD.[15] Pairs of GM masks (Figure 2) were used as seed and target areas (step-size = 0.1 mm, maximum angle between steps = 10°, maximum harmonics order = 8, termination criteria: CSD fibre-orientation distribution amplitude was < 0.1, number of tracks: 3000). Tracts were then mapped back on TDI images and their trajectory assessed for anatomical accuracy by MP.

e) *Generation of WM tract of interest (TOI) masks:* All tracts obtained in (c) were registered to MNI space using the inverse of the transformation described in (c). Where a voxel was included in a tract in ≥ 50% of controls[S5] it was also included in the final TOI (Figure 2). Processing time for the pipeline to create TOI tracts was about 12 hours per subject.

Network based analysis: Quantification of motor network WM tract Fractional Anisotropy (FA), Magnetisation Transfer Ratio (MTR) and volume

FA, MTR and normalized volume was computed for each Tract of Interest (TOI) for all MS subjects as follows:

a) *Registration of each tract to FA maps*: TOI masks were co-registered from atlas space to each subject native diffusion space as previously described[S4] using the native b0 image as target of the non-linear registration.

b) *Registration of each tract to T1 volumetric images*: TOI maps were non-linearly co-registered from MNI space to each subject volumetric T1 image using nifty-reg.[S3]

c) *Registration of MTR maps to T1-weighted volumetric images*: MT on and MT off images for each subject were affine registered to their corresponding T1-weighted volume image. MTR was calculated for each voxel as S(0)-S(RF)/S(0), where S(0) and S(RF) are the signal intensities without and with the application of the off-resonance pulse.

d) *Quantification of FA, MTR and volume*: To ensure that only WM voxels were included in TOI masks, SPM8 (www.fil.ion.ucl.ac.uk/spm/software/spm8) was used to segment the lesion-filled T1-weighted volume images to create a subject-specific WM mask. This mask was registered to diffusion space using a non-linear registration.[S3,S4] Each TOI was then intersected with the WM mask and then used to compute FA, MTR and volume values inside each tract. TOI volume was normalized using intracranial volume data computed using segmented T1 images.

SUPPLEMENTARY RESULTS

MRI AND EDSS ASSOCIATIONS IN EDSS > 3.5 AND EDSS <= 3.5 SUBGROUPS

Twenty seven subjects had an EDSS score ≤3.5 and 44 an EDSS > 3.5. Assessing correlations of EDSS with composite NE in these subgroups, these remained significant in both the group with an EDSS ≤3.5 (R-square 0.2) and with EDSS >3.5 (R-square 0.4). The lower R-square values observed in these subgroups compared with the whole MS cohort is as expected, consistent with the smaller range of MRI and EDSS measures seen in the subgroups. A comparison of the residual variance in the two subgroups provided evidence that composite NE prediction of EDSS is better in the EDSS > 3.5 group ($P<0.001$). Neither disease duration nor any other variable predicted EDSS independently of composite NE in separate models for the two sub-groups.

SUPPLEMENTARY REFERENCES

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S4. Muhlert N, Sethi V, Schneider T, et al. Diffusion MRI-based cortical complexity alterations associated with executive function in multiple sclerosis. Journal of magnetic resonance imaging : JMRI 2013;38:54-63.

S5. Aron AR, Behrens TE, Smith S, Frank MJ, Poldrack RA. Triangulating a cognitive control network using diffusion-weighted magnetic resonance imaging (MRI) and functional MRI. The Journal of neuroscience : the official journal of the Society for Neuroscience 2007;27:3743-3752.