# supplemental information (SI)

Delayed access to conscious processing in Multiple Sclerosis: Reduced cortical activation and impaired structural connectivity

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**SI REFERENCES**

# SI Method

## MRI data acquisition

DTI scans were collected using a single-shell with 32 independent directions with non-collinear diffusion gradients (b  =  1000 s/mm2) and a non-diffusion-weighted image (b  =  0 s/mm2) (repetition time (TR) = 7200 ms, echo time (TE) = 90 ms, voxel size = 1.9 × 1.9 × 2.0 mm3, FOV = 240 mm, matrix = 128 x 128, 54 axial sections, without intersection gap, acquisition time (TA) = 12:46 min). For task-based fMRI, we used a T2\*-weighted (T2\*W), blood oxygen level dependent (BOLD) sensitized multiband (MB) echo planer imaging (EPI) sequence with a factor of 2 to increase the temporal resolution (MB = 2, TR = 2000 ms, TE = 30 ms, 60 slices, voxel size = 2 × 2 × 2 mm3, FOV = 224 × 224 mm2, matrix size = 112 × 112, TA = 15:32 min). T2-weigthed imaging parameters were given as following; TR = 2800 ms, TE = 90 ms, 43 slices, voxel size = 0.5 × 0.5 × 3.0 mm3, no gap, matrix = 256 × 256, FOV = 240 mm, TA = 3:46 min and 3D T1-W MPRAGE parameters were; TR = 1800 ms, TE = 2.3 ms, flip angle = 8 °, FOV = 250 mm, matrix = 288 × 288, TA = 3:39 min. Two-dimensional FLAIR images were obtained using the following parameters; TR = 9 ms, TE = 0.09 ms, inversion time = 2.5 s, flip angle = 150 °, 13 slices, 3 mm slice thickness, matrix = 162 × 162, TA = 2:08 min.

## Cortical thickness

Then, we used Freesurfer (www.surfer.nmr.mgh.harvard.edu) to perform a cross-sectional vertex-wise comparison of cortical thickness between pwMS and controls using general linear model (GLM) controlling for age and sex. In a second model, the association between cortical thickness and the delay in access to conscious processing was calculated, again we corrected for age and sex in the GLM. Vertex-wise results were corrected by the false discovery rate (FDR) at a threshold of p < 0.05.

## Structural connectivity

We used the DTI images to perform probabilistic tractography with MRTRIX3 (J.-D. Tournier et al., 2019) as described in Besson et al (2014)in a recent paper from our group (Has Silemek et al., 2020). Briefly, the distortion of the DTI images were corrected by *eddy* in FSL that corrected the subject motion and eddy current in the gradient coils and skull stripping was applied and then the fractional anisotropy and mean diffusivity were calculated using tensor fitting in the FSL software (Behrens, Berg, Jbabdi, Rushworth, & Woolrich, 2007).

Fiber orientation distribution (FOD) at the FA values higher than 0.7 determined the response function for the accurate estimation of FOD during constrained spherical deconvolution (J. D. Tournier, Calamante, & Connelly, 2007). Then, probabilistic tractography algorithm (Behrens et al., 2007) generating 150,000 fibers with a minimum threshold of 20 mm was used to build the fibers (default parameters: step size: 0.2 mm, minimum radius of curvature: 1 mm, FOD cut-off: 0.1). Specification of the seeds was done by all voxels of 1 mm dilated white matter masks. Using the edge of mask and predefined FA and FOD thresholds, the tracking of seeds was limited. Then, after the FA values estimation at each point of the fiber, the average FA for each fiber was calculated.

We used the average FA to constructed weighted connectivity matrices between 160 nodes (80 cortical regions per hemisphere) based on the Destrieux atlas. Besides these anatomical labels, we labeled the nodes according to their location in the visual processing pathway. Based on the two stream hypothesis; and only for descriptive purpose, we labeled nodes as belonging to the visual system and eventually to one of the two streams. Strength as a measure of regional structural connectivity was calculated as the sum of weighted connections within and between each node for each subject.

## Tract based spatial statistics

We used tract-based spatial statistics (TBSS) to compare the whole brain fiber bundles between the groups by enabling the statistical evaluation of variations in the major white matter pathways voxel-by-voxel (Smith et al., 2006). FA maps of all subjects were registered and aligned to common space by nonlinear registration and then mean FA skeleton was calculated and thinned. Voxel-wise comparison included 10000 permutation tests controlling by family wise error (FWE) correction with the threshold free cluster enhancement (TFCE) at a certain threshold (p < 0.05). We used John Hopkins University White Matter Atlas to recognize the tracts(Hua et al., 2008). We then applied linear mixed models to compare the mean FA of region of interests (ROI) that were drawn based on TBSS analysis adjusting by age and sex. Likewise, mixed models controlling by age and sex were applied to extract the correlations between the mean FA of ROIs and behavioral inflection point.

# SI Results



**Figure S1. History of optic neuritis and visual assessments, VEP and OCT.** Figure indicates the difference between affected and unaffected eyes in the subgroup of pwMS with a history of optic neuritis (n=13).

**Table S1.**  **Optic neuritis, visual assessments, OCT and VEP.** Among the 13 pwMS with a history of optic neuritis, we compared outcomes between affected and unaffected eyes.

|  |  |  |  |
| --- | --- | --- | --- |
| **Optic neuritis** | **affected eye** | **unaffected eye** | **p value** |
| n | 13 | 13 |  |
| VA5m | 0.86 (0.32) | 0.82 (0.23) | 0.731 |
| Sloan2.5 | 18.38 (10.11) | 23.77 (9.48) | 0.174 |
| AULCSF | 1.05 (0.27) | 1.14 (0.21) | 0.337 |
| VEP | 131 (17.4) | 126.87 (17.14) | 0.609 |
| RNFL | 10.86 (2.98) | 10.86 (3.38) | 1 |
| GCIPL | 0.29 (0.032) | 0.26 (0.027) | 0.777 |

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**Figure S2. Associations between accuracy obtained during fMRI experiment and clinical and visual assessments.** No association between accuracy and visual acuity, VEP, loss of retinal integrity or disability measurements in MS and healthy controls.

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**Figure S3. Cortical thickness and its association with inflection point (A)** Reduced cortical thickness in people with Multiple Sclerosis (pwMS) compared to healthy controls. FDR corrected regions illustrated in yellow. Right (upper) and left (lower) medial, lateral, inferior and superior views **(B**) Beta values of the interaction between cortical thickness and inflection point (before FDR correction). No significant correlation was found after correcting for multiple testing.

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**Figure S4.** **Global structural connectivity is associated with task performance.** **(A)** Group difference of global structural connectivity and **(B)** its correlation with the inflection point that is the individual time needed to access consciousness in pwMS and healthy controls.



**Figure S*5*. The NBS determined subnetwork of correlations between the individual inflection point and structural connectivity in people with Multiple Sclerosis (pwMS)**. Colors indicate the association of nodes with functional or anatomical subnetworks based on location. Edge widths represents r values.

**Table S2. Regions and subdivisions that are correlated with the behavioral inflection point either in patients or controls.** Color code indicates the location of MNI brain regions that are shown any kind of correlation with the behavioral inflection point within the groups. These regions are illustrated in Figure S5.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Nodes** | **Subdivision** | **x.mni** | **y.mni** | **z.mni** | **Color code** |
| Left.Thalamus.Proper | Sub cortical gray matter | -9 | -17 | 6 | yellow |
| Right.Thalamus.Proper | Sub cortical gray matter | 10 | -19 | 6 | yellow |
| ctx\_lh\_G\_and\_S\_occipital\_inf | Primary visual | -39.27 | -81.242 | -11.977 | Burgundy |
| ctx\_lh\_G\_front\_middle | Dorsal stream | -37.27 | 29.7 | 31.553 | cadetblue3 |
| ctx\_lh\_G\_front\_sup | Dorsal stream | -9.03 | 21.65 | 51.327 | cadetblue3 |
| ctx\_lh\_G\_insular\_short | Insula | -35.043 | 8.586 | -2.554 | Navy-blue |
| ctx\_lh\_G\_occipital\_middle | Primary visual | -39.239 | -82.374 | 11.232 | Burgundy |
| ctx\_lh\_G\_occipital\_sup | Primary visual | -14.847 | -86.955 | 26.707 | Burgundy |
| ctx\_lh\_G\_precentral | Frontal | -41.773 | -9.72 | 51.554 | red |
| ctx\_lh\_G\_precuneus | Dorsal stream | -7.562 | -58.501 | 45.07 | cadetblue3 |
| ctx\_lh\_Pole\_occipital | Primary visual | -17.721 | -97.276 | -5.706 | Burgundy |
| ctx\_lh\_S\_circular\_insula\_ant | Insula | -27.738 | 22.278 | -9.516 | Navy-blue |
| ctx\_lh\_S\_circular\_insula\_inf | Insula | -39.502 | -13.872 | -7.846 | Navy-blue |
| ctx\_lh\_S\_circular\_insula\_sup | Insula | -34.484 | 5.133 | 11.951 | Navy-blue |
| ctx\_lh\_S\_front\_middle | Dorsal stream | -25.968 | 43.597 | 18.904 | cadetblue3 |
| ctx\_lh\_S\_front\_sup | Dorsal stream | -22.711 | 17.928 | 43.159 | cadetblue3 |
| ctx\_lh\_S\_occipital\_ant | Primary visual | -41.7 | -68.154 | 0.278 | Burgundy |
| ctx\_lh\_S\_parieto\_occipital | Dorsal stream | -16.599 | -67.45 | 22.655 | cadetblue3 |
| ctx\_lh\_S\_precentral.inf.part | Frontal | -43.352 | 2.957 | 27.034 | red |
| ctx\_lh\_S\_precentral.sup.part | Frontal | -26.797 | -9.499 | 50.401 | red |
| ctx\_rh\_G\_and\_S\_occipital\_inf | Primary visual | 42.104 | -76.432 | -11.482 | Burgundy |
| ctx\_rh\_G\_front\_middle | Dorsal stream | 38.204 | 29.401 | 30.849 | cadetblue3 |
| ctx\_rh\_G\_front\_sup | Frontal | 9.28 | 22.781 | 52.457 | red |
| ctx\_rh\_G\_insular\_short | Insula | 36.318 | 9.454 | -2.202 | Navy-blue |
| ctx\_rh\_G\_occipital\_middle | Primary visual | 40.033 | -79.453 | 12.821 | Burgundy |
| ctx\_rh\_G\_occipital\_sup | Primary visual | 18.948 | -85.935 | 31.252 | Burgundy |
| ctx\_rh\_G\_precentral | Frontal | 40.651 | -8.156 | 53.418 | red |
| ctx\_rh\_G\_precuneus | Dorsal stream | 7.723 | -59.505 | 46.822 | cadetblue3 |
| ctx\_rh\_Pole\_occipital | Primary visual | 17.394 | -94.985 | -3.658 | Burgundy |
| ctx\_rh\_S\_circular\_insula\_ant | Insula | 29.668 | 23.312 | -7.027 | Navy-blue |
| ctx\_rh\_S\_circular\_insula\_inf | Insula | 41.392 | -12.95 | -7.87 | Navy-blue |
| ctx\_rh\_S\_circular\_insula\_sup | Insula | 34.968 | 5.708 | 11.641 | Navy-blue |
| ctx\_rh\_S\_front\_middle | Dorsal stream | 28.296 | 40.93 | 22.288 | cadetblue3 |
| ctx\_rh\_S\_front\_sup | Frontal | 22.69 | 18.148 | 44.827 | red |
| ctx\_rh\_S\_occipital\_ant | Primary visual | 42.996 | -65.226 | 4.085 | Burgundy |
| ctx\_rh\_S\_parieto\_occipital | Dorsal stream | 17.959 | -65.668 | 24.804 | cadetblue3 |
| ctx\_rh\_S\_precentral.inf.part | Frontal | 42.614 | 4.307 | 27.246 | red |
| ctx\_rh\_S\_precentral.sup.part | Frontal | 26.621 | -8.68 | 51.56 | red |

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