## SUPPLEMENTARY MATERIAL

## METHODS

## Magnetic resonance imaging (MRI) analysis

**MRI protocol**

MRI was performed for all subjects using a 3 Tesla scanner (MAGNETOM Trio Time, Siemens, Erlangen, Germany). This study protocol included a 3D T1-weighted magnetization prepared rapid acquisition gradient echo (MPRAGE) sequence (repetition time (TR) = 1900 ms, echo time (TE) = 3.03 ms, isotropic resolution 1x1x1 mm), a 3D T2-weighted fluid-attenuated inversion recovery (FLAIR) sequence (TR = 6000 ms, TE = 388 ms, isotropic resolution 1x1x1 mm), and a diffusion tensor imaging (DTI) sequence (TR = 7500 ms, TE = 86 ms, matrix 96x96, slice thickness 2.3 mm, 64 noncollinear directions, b-value=1000 s/mm2) of the brain.

All patient MRIs were acquired on the same day as the clinical examination, except for three sessions, where there were time intervals of one day (n=2) and 30 days (n=1) apart.

## Measurement of the LGN using the MAGeT brain algorithm

The Multiple Automatically Generated Templates (MAGeT) brain algorithm [1,2] was used to segment the entire thalami and the different thalamic nuclei on MPRAGE MRI scans. MAGeT uses an atlas derived from manually segmented serial histological data, containing delineation of the thalamic nuclei, as per Hirai and Jones [3]. It first customizes the atlas to a subset of participants, representative of the entire study population, using a nonlinear registration scheme.

In our study, this representative subset was chosen in a manner consistent with best practices for the algorithm [4], according to age, sex and - for patients - number of optic neuritis (ON). It consisted of 23 NMOSD patients with the following characteristics: 21/23 (91.3%) women, mean age: 48.7±15.9 years, all white, median number of attacks 3 (range 1-22), 15/23 (65.2%) with previous ON, median number of ON 1 (range: 1-14) and eight healthy controls with the following characteristics: 6/8 (75%) women, mean age: 39.8±16.1 years, all white.

We included MRI sessions from all different time-points, according to the number of available MRIs at the different years (for patients: n=6 from baseline, n= 6 from year 1, n=5 from year 2, n=4 from year 3 and n=2 from year 4; for HC: n=4 from baseline, n=3 from year 1 and n=1 from year 2). This newly segmented subset of MRIs acted as a template library for the remaining participants, to correct for the neuroanatomical variability of our study population and to average different sources of random error prior to the final segmentation [5].

**Diffusion tensor imaging (DTI) and probabilistic tractography of the optic radiations**

Diffusion tensors on the DTI images were fitted by a linear-least square approach. MRtrix package 3.0 (J-D Tournier; Brain Research Institute, Melbourne, Australia) was used to perform probabilistic tractography from seed to target mask [6]. Fiber orientation distribution was estimated with constrained spherical deconvolution and mapped with a maximum harmonic order of 6. The OR reconstruction pipeline was modified from the Martinez-Heras et al. and Lim et al. pipeline. The Juelich probabilistic atlas was used to generate binary masks of the LGN as the seed region of interest (ROI) and the primary visual cortex (V1) as the target ROI. For binary exclusion masks, a midline sagittal exclusion plane, a termination coronal plane 20 mm posterior to the temporal pole, and a gray matter segmentation mask were created in the 3D coordinate system of the Montreal Neurological Institute (MNI-152). These were subsequently registered to individual DTI space, serving as a binary exclusion ROI for tractography. Ten thousand unidirectional streamlines from the LGN to V1 were generated (fractional anisotropy (FA) threshold: 0.1; curvature threshold: 25%; step size: 0.2 mm) for each OR. Streamlines were thresholded for 25% of the maximum value. Resulting fibers were transferred to the Vistalab environment (vistalab.stanford.edu/, Vistalab, Stanford University, Stanford, CA) to compute tract profiles of weighted mean DTI values of FA, mean diffusivity (MD), radial diffusivity (RD), and axial diffusivity (AD) at 50 equally spaced positions. The mean of these 50 positions was used in the analysis.

**Optical coherence tomography scanning protocol**

Retinal imaging was performed using a Heidelberg Engineering Spectralis spectral domain optical coherence tomography (OCT; Heidelberg Engineering, Heidelberg, Germany), with ART (automatic real-time) function for image averaging. We did not use pupil dilation.

We report the OCT acquisition settings and scanning protocol according to the APOSTEL recommendations [7]:

The peripapillary retinal nerve fiber layer (pRNFL) was measured using 3.4-mm ring scans around the optic nerve head (12°, 1536 A-scans, 9≤ ART ≤100). The combined ganglion cell and inner plexiform layer (GCIPL) volume was measured using a 5-mm diameter donut cylinder around the fovea from a macular volume scan (25°x30°, 61 vertical B-scans, 768 A-scans per B-scan, ART=15). Quality control of the scans was performed using the OSCAR-IB Criteria [8] and a total of 13 ring scans and 15 macula scans were rejected.

The segmentation of the pRNFL and the intraretinal layers in the macular scan was performed using the software “SAMIRIX”, as previously described by our group [9]. All segmentations were manually corrected by one experienced rater (F.C.O.).

**RESULTS**

**Longitudinal course of LGN volume in four patients with and four patients without new ON during follow-up**

After showing a reduction in total LGN volume in four patients that suffered a new ON episode during the study, we also examined the course of LGN volume in four patients that did not show new ON episodes during follow-up. We chose these patients to be as well matched as possible to the four patients with new ON. The characteristics of these eight patients are summarized in the supplementary table S1.

**Supplementary table S1: Characteristics of the four patients with new ON during follow-up and the four matched patients (“negative controls”) without new ON**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Patients with new ON during follow-up** | Age, y | Sex | Race | Prior ON  | Number of prior ON | Other auto-immune disease  | SLE | Treatment |
| P01 | 32.0 | woman | caucasian | yes | 2 | no | no | yes (with change)\* |
| P02 | 35.6 | woman | caucasian | yes | 1 | yes | yes | yes (with change)\* |
| P03 | 20.9 | woman | caucasian | yes | 4 | yes | no | yes (with change)\* |
| P04 | 51.4 | man | caucasian | yes | 2 | no | no | yes (no change)\* |
| **Summary of P01-P04** | **35 ± 12.6** | **3 women: 1 man** | **4/4 caucasian** | **4/4 NMO-ON** | **Median 2** | **2 yes: 2 no** | **1 yes: 3 no**  | **4/4 yes (3/4 with a treatment change during follow-up)** |
| **Matched patients without new ON during follow-up** |  |  |  |  |  |  |  |  |
| CP01 | 29.8 | woman | caucasian | yes | 2 | yes | yes | yes (with change)\* |
| CP02 | 38.6 | woman | caucasian | yes | 1 | no | no | yes (with change)\* |
| CP03 | 20.9 | woman | caucasian | yes | 1 | yes | no | yes (no change)\* |
| CP04 | 68.4 | man | caucasian | yes | 1 | no | no | yes (no change)\* |
| **Summary of CP01-CP04** | **39.4 ± 20.6** | **3 women: 1 man** | **4/4 caucasian** | **4/4 NMO-ON** | **Median 1** | **2 yes: 2 no** | **1 yes: 3 no**  | **4/4 yes (2/4 with a treatment change during follow-up)** |

**Legend table S1**

\*: Most patients had some change in their immunosuppressive treatment during follow-up: P01 from rituximab to azathioprine (between V0 and V1), P02: from methotrexate to methotrexate and rituximab (between V0 and V1), P03: from azathioprine to prednisolone (between V0 and V1) and from prednisolone to mycophenolate mofetil (between V1 and V2), CP01: from rituximab to azathioprine (between V2 and V3), and CP02: from azathioprine to rituximab and from rituximab to mycophenolate mofetil (between V0 and V1). P04, CP03 and CP04 had no treatment change during the entire follow-up period (the first two had azathioprine and the last rituximab).

Abbreviations: NMO-ON: patients with neuromyelitis optica and prior optic neuritis, ON= optic neuritis, SLE= systemic lupus erythematodes, y= years

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