Highlights

- The anterior, lateral, medial and posterior thalamic nuclei and tracts were automatically segmented by using a histopathological atlas.

- Changes within thalamic tracts are equally important as whole-brain lesion load in predicting cognitive functioning in patients with MS.

- Of the neuropsychiatric measures, disinhibition is mainly determined by lesion load within thalamic tracts while changes in agitation can be explained by thalamic volume.
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Cognition and disinhibition in multiple sclerosis: impact of MRI changes within thalamic tracts

Submitted

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Chapter 4.2

Abstract

Objective
To investigate groups of thalamic nuclei (anterior, posterior, medial, lateral) and the integrity of their associated tracts with regard to cognitive and neuropsychiatric symptoms in multiple sclerosis (MS) patients.

Methods
Conventional MRI and diffusion tensor imaging (DTI) was acquired in 73 MS patients and 18 healthy controls. Extensive neuropsychological testing was performed and neuropsychiatric symptoms were assessed. Based on a histological atlas, the thalamus was parcellated into an anterior, lateral, medial and posterior part. The associated tracts were obtained by probabilistic tractography. Integrity of the tracts was expressed by fractional anisotropy (FA) and mean diffusivity (MD). Lesion load (LL) within the tracts was also determined. Multivariate regression analysis was performed to define the most important predictor for cognitive and neuropsychiatric symptoms.

Results
In all four thalamic tracts, MD and LL correlated with cognitive performance in MS patients. For FA, changes in the anterior and posterior tract of the thalamus related to cognition. Most important predictors for cognitive functioning were whole brain T2 LL (standardized $\beta = -0.36$), MD within the thalamic tracts (standardized $\beta = -0.31$) and educational level (standardized $\beta = 0.22$); these factors explained 48.1% of the variance in cognitive performance in MS. With regard to neuropsychiatric symptoms: disinhibition could be explained for 18.8% by LL within the thalamic tracts, while thalamic volume explained 12.2% of the variance in agitation.

Conclusions
Changes within thalamic tracts are equally important as T2 lesion load in predicting cognitive functioning in patients with MS, while neuropsychiatric functioning (disinhibition) is mainly determined by lesion load within thalamic tracts.
**Introduction**

Thalamic pathology is frequently present in patients with multiple sclerosis (MS). Neuronal loss and demyelination were described in thalamic tissue of MS patients (1,2) and thalamic atrophy (3,4), changes in N-acetylaspartate (5) (a marker for neuronal integrity) and changes in diffusion tensor imaging (DTI) (6) were reported using magnetic resonance imaging (MRI). These thalamic abnormalities are highly correlated with conversion to clinically definite MS, physical disability and cognitive impairment (3,6–8).

Recently it was shown that changes in functional connectivity of the thalamus, changes in diffusivity of the thalamus, and thalamic volume loss were present especially in the most severely cognitively impaired MS patients (9–11).

Anatomically, the thalamus can be subdivided into a number of different thalamic nuclei, each with their own specific (sub) cortical output and input areas (12,13). This implies that damage to specific thalamic nuclei and/or their associated tracts should result in disparate symptoms. To increase our understanding of the negative effects of thalamic pathology in MS, it might be necessary to discriminate between the different nuclei of the thalamus and their associated tracts, rather than regarding the thalamus as a homogenous solitary structure.

Therefore, the aim of the current study was to examine the anterior, posterior, medial and lateral thalamic nuclei and the integrity of their associated white matter tracts using DTI, in order to unravel their explanatory power with regard to cognitive impairment and neuropsychiatric symptoms in patients with MS.

**Methods**

Part of the data from this study has been previously published to show the extent of thalamic damage in MS, as well as its clinical significance (6).

**Participants**

All patients were diagnosed with clinically definite MS according to the revised McDonald Criteria (14) and were relapse-free and off steroid treatment for at least 30 days prior to examination. Disease type (relapsing-remitting MS or secondary-progressive MS) and disease duration were determined by clinical assessment. Disease severity was determined based on the Expanded Disability Status Scale (EDSS).

Healthy controls (HC) were also included. Patients and HCs were free from any history of
(additional) neurological disease, psychiatric disturbances, alcohol or drug abuse and all met the safety criteria to undergo an MRI scan. The Institutional Ethics Review Board approved the study protocol and all subjects gave written informed consent prior to participation.

**Neuropsychological evaluation**

An internationally renowned cognitive test battery, the MACFIMS (15), was administered. In short, the following cognitive domains were tested:

- **Verbal learning and memory** *(California Verbal Learning Test, 2nd edition (CVLT–20)) (16).*
- **Visuospacial memory** *(Brief Visual Memory Test - Revised (BVMT-R)) (17).*
- **Information processing speed** *(Symbol Digit Modalities Test (SDMT)) (18).*
- **Cognitive processing speed and attention** *(Paced Auditory Serial Addition Test (PASAT)) (19).*
- **Executive functioning** *(Delis-Kaplan Executive Function System Sorting Test (D-KEFS)) (20).*
- **Verbal fluency and memory retrieval** *(Controlled Oral Word Association Test (COWAT)) (21).*
- **Visuospatial ability** *(Judgment of Line Orientation Test (JLO)) (22).*

**Neuropsychiatric symptoms**

The neuropsychiatric inventory (NPI) is a structured interview that explores the following 10 symptom domains: delusions, hallucinations, agitation, depression, anxiety, euphoria, apathy, disinhibition, irritability and aberrant motor behavior. Scores were calculated based on the severity of the symptoms (0 [mild] to 3 [severe]) and the frequency (0 [none] to 4 [daily]) (23).

**Brain volumes, WM lesions and thalamic volume**

All subjects underwent MR imaging on a 3T General Electric Signa Excite HD 12.0 Twin Speed 8-channel scanner. Grey matter (GM) and white matter (WM) volumes were determined using the 3D T1-weighted fast spoiled gradient recalled echo with magnetization-prepared inversion recovery (IR-FSPGR, repetition time (TR) 5.9 ms, echo time (TE) 2.8 ms, inversion time (TI) 900 ms, flip angle = 10°, 25.6 × 19.2 cm² field-of-view (FOV), 256 × 256 matrix with
phase FOV = 0.75, 128 slices of 1.5 mm) images using SIENAX 2.6, which is part of the FSL toolbox (http://www.fmrib.ox.ac.uk/fsl). Prior to using the T1-weighted images for subsequent analysis, they were modified using an in-house developed in-painting technique to mitigate the impact of WM lesions on tissue segmentation. WM lesions were outlined on the 2D fluid-attenuated inversion recovery (FLAIR) images (TR 8500 ms, TE 120 ms, TI 2100 ms, flip angle = 90°, echo train length (ETL) = 24, FOV 25.6 × 19.2 cm², 256 × 256 matrix with phase FOV = 0.75, 48 slices of 3 mm, no gap) using a semi-automated edge detection contouring/thresholding technique previously described (24). Thalamus volumes were calculated using FIRST and corrected for head size using the scaling factor from SIENAX.

**Thalamic parcellation**

The anatomical information to subdivide the thalamus into different parts was obtained from a high-resolution 3D-atlas of the thalamic nuclei (25). This atlas was constructed by combining several stacks of histologically processed (and delineated) brain sections. The average delineations were mapped onto a 1 × 1 × 1 mm³ MNI152 standard space, constituting 38 labels per thalamus each representing different thalamic nuclei or structures. This atlas was used to get subject-specific segmentations of the thalamic nuclei.
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Since the contrast and spatial resolution of MRI are limited compared to histopathology, the structures in the atlas were first merged into four coarse thalamic sections (25,26) (see also Figure 1).

1. **Medial part:** consisting of the mediodorsal, medioventral, paraventricular, habenular and the subparafascicular nuclei;

2. **Posterior part:** consisting of the pulvinar, lateral posterior, suprageniculate, limitans and posterior nuclei;

3. **Lateral part:** consisting of the ventral nuclei;

4. **Anterior part:** consisting of the lateral dorsal and anterior nuclei.

Subsequently, FNIRT (part of FSL) was used to nonlinearly map the T1-weighted image of each subject to MNI-space. The cost-function of the nonlinear registration was masked by the inverse lesion mask to minimize the influence of lesions. The subject-specific parcellation was obtained by mapping the labels of the thalamic sections into subject-space using the inverse warp field. The volume of all four thalamic sections was calculated and corrected for head size.

**Probabilistic tractography, thalamic tract integrity and thalamic tract lesions**

Diffusion-weighted images (2D echo-planar imaging (EPI), TR 8.6 sec, TE 90.9 ms, flip angle = 90°, ETL = 1, FOV 32 × 24 cm² (96 × 96 matrix), 3.33 × 3.33 mm², slice thickness 3 mm no gap, 1 repetition, b = 800 s/mm², using 1 volume without directional weighting (b0) and 15 volumes with non-collinear diffusion gradients) were corrected for movement and eddy current distortion using the Diffusion Toolbox (FDT; also part of FSL). The diffusion tensor was fitted, from which the fractional anisotropy (FA) and mean diffusivity (MD) measures were calculated.

The tracts associated with the individual thalamic regions were then localized. As tractography in the presence of MS pathology might lead to unreliable results, this was done by means of a probabilistic atlas based on an independent sample of 27 HCs (18 females, mean age: 43.2 years, standard deviation: 16.0 years).

In short, for each subject and tract separately, the weighted average FA and the weighted average MD values inside the normal-appearing white matter (NAWM) of each tract, as well as the lesion load (LL) within the tract, was computed using the atlas probability values as a weighting factor. Values within the center of each tract have a higher certainty that the area accurately belonged to that particular tract compared to values located at the border.
Thalamic tracts, cognition and disinhibition in MS

of the tract. Using weighted scores therefore emphasized all values within the center of the tract. In order to only include NAWM, the weighting factors of voxels belonging to GM, CSF or lesions were set to zero using the earlier derived SIENAX and lesion segmentations (see Figure 2 and Supplementary Figure 1 for the thalamic projections). For the complete pipeline see Supplementary Materials.

**Statistical analysis**

Statistical analyses were performed in SPSS 20.0 (Chicago, IL, USA). When variables were normally distributed, an independent t-test was performed to describe differences in demographic, neuropsychological and neuroimaging variables between MS patients and HCs. When variables were not normally distributed, a Mann-Whitney test was used. Spearman correlations were calculated and corrected for multiple comparisons using a Bonferroni correction. Multivariate regression analysis was performed to identify the most important imaging predictor(s) for cognitive and neuropsychiatric functioning. For this purpose the following variables were calculated: mean thalamic tract FA, mean thalamic tract MD and mean thalamic tract lesion volume (a mean value for the four tracts combined). Additionally, an average cognition score was calculated based on the Z-scores of patients on the different
Supplementary Figure 2. The four different thalamic projections originating from the anterior (ANT), posterior (POST), medial (MED) and lateral (LAT) parts of the thalamus. Projections from the left and right thalamus are shown.

cognitive tests. Whole brain LL and LL within the tracts were log-transformed to assure normality. P-values ≤ 0.05 were considered significant. The left and right thalamus variables were averaged due to $r$ values ranging from 0.79–0.91.

**Results**

Seventy-three patients (51 females) and 18 HCs (11 females) participated in the study. In Table 1, the demographic data of MS patients and HCs are provided. Forty-eight patients had a relapsing-remitting disease course, the other 25 patients had secondary-progressive MS. Patients and controls did not differ with regard to sex, age and race. Educational level was higher in the HC group ($p = 0.022$).

Patients with MS performed worse on all cognitive tests except for the judgment of line
**Table 1.** Demographic, conventional MRI, neuropsychological and personality characteristics.

<table>
<thead>
<tr>
<th></th>
<th><strong>MS (n = 73)</strong></th>
<th><strong>HC (n = 18)</strong></th>
<th><strong>P-value</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td>46.51 ± 9.00</td>
<td>42.11 ± 11.54</td>
<td>0.083</td>
</tr>
<tr>
<td><strong>Educational level</strong></td>
<td>14.00 – 12.50 – 16.00</td>
<td>16.00 – 13.50 – 18.00</td>
<td>0.022</td>
</tr>
<tr>
<td><strong>Male/female</strong></td>
<td>22/51</td>
<td>8/10</td>
<td>0.475</td>
</tr>
<tr>
<td><strong>Caucasian/other</strong></td>
<td>62/11</td>
<td>15/3</td>
<td>0.567</td>
</tr>
<tr>
<td><strong>EDSS</strong></td>
<td>3.50 ± 2.00 – 5.50</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Disease Duration</strong></td>
<td>11.55 ± 7.53</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>NGMV (mL)</strong></td>
<td>738.24 ± 56.35</td>
<td>757.42 ± 42.36</td>
<td>0.180</td>
</tr>
<tr>
<td><strong>NWMV (mL)</strong></td>
<td>714.63 ± 45.96</td>
<td>753.69 ± 35.26</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>NTV (mL)</strong></td>
<td>13.34 ± 1.82</td>
<td>15.66 ± 1.55</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td><strong>CVLT–2 (total learning)</strong></td>
<td>53.00 ± 43.50 – 61.00</td>
<td>69.00 ± 61.75 – 70.25</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td><strong>BVMT-R (total learning)</strong></td>
<td>19.00 ± 15.00 – 24.00</td>
<td>27.00 ± 22.75 – 31.25</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td><strong>SDMT</strong></td>
<td>50.00 ± 38.00 – 59.50</td>
<td>63.50 ± 56.00 – 74.25</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td><strong>PASAT 2 s</strong></td>
<td>30.00 ± 25.00 – 37.00</td>
<td>37.00 ± 31.75 – 44.25</td>
<td>0.004</td>
</tr>
<tr>
<td><strong>D-KEFS Sorting</strong></td>
<td>10.00 ± 7.50 – 11.00</td>
<td>12.00 ± 9.75 – 13.00</td>
<td>0.004</td>
</tr>
<tr>
<td><strong>JLO</strong></td>
<td>24.00 ± 19.00 – 27.00</td>
<td>24.00 ± 22.00 – 26.00</td>
<td>0.635</td>
</tr>
<tr>
<td><strong>COWAT</strong></td>
<td>33.00 ± 25.00 – 42.50</td>
<td>40.00 ± 32.75 – 45.25</td>
<td>0.022</td>
</tr>
<tr>
<td><strong>NPI delusion</strong></td>
<td>0.00 ± 0.00 – 0.00</td>
<td>0.00 ± 0.00</td>
<td>1.000</td>
</tr>
<tr>
<td><strong>NPI hallucination</strong></td>
<td>0.03 ± 0.23 – 2.00</td>
<td>0.00 ± 0.00</td>
<td>0.619</td>
</tr>
<tr>
<td><strong>NPI agitation</strong></td>
<td>1.29 ± 2.28 – 8.00</td>
<td>0.00 ± 0.00</td>
<td>0.004</td>
</tr>
<tr>
<td><strong>NPI depression</strong></td>
<td>1.55 ± 2.88 – 12.00</td>
<td>0.00 ± 0.00</td>
<td>0.002</td>
</tr>
<tr>
<td><strong>NPI anxiety</strong></td>
<td>0.70 ± 1.67 – 8.00</td>
<td>0.00 ± 0.00</td>
<td>0.045</td>
</tr>
<tr>
<td><strong>NPI euphoria</strong></td>
<td>0.71 ± 2.19 – 12.00</td>
<td>0.00 ± 0.00</td>
<td>0.099</td>
</tr>
<tr>
<td><strong>NPI apathy</strong></td>
<td>1.29 ± 2.76 – 12.00</td>
<td>0.00 ± 0.00</td>
<td>0.016</td>
</tr>
<tr>
<td><strong>NPI disinhibition</strong></td>
<td>0.81 ± 2.01 – 12.00</td>
<td>0.00 ± 0.00</td>
<td>0.016</td>
</tr>
<tr>
<td><strong>NPI irritability</strong></td>
<td>1.56 ± 2.75 – 12.00</td>
<td>0.00 ± 0.00</td>
<td>0.002</td>
</tr>
<tr>
<td><strong>NPI aberrant motor</strong></td>
<td>0.25 ± 1.25 – 8.00</td>
<td>0.00 ± 0.00</td>
<td>0.384</td>
</tr>
</tbody>
</table>

Abbreviations: MS = multiple sclerosis; SD = standard deviation; IQR = inter quartile range; EDSS = Expanded Disability Status Scale; BDI = Beck Depression Inventory; FSS = fatigue severity scale; NGMV = normalized gray matter volume; NWMV = normalized white matter volume; NTV = normalized thalamic volume; CVLT = California Verbal Learning Test - 2nd edition; BVMT-R = Brief visual memory test - revised; SDMT = symbol digit modalities test; PASAT = paced auditory serial attention test; D-KEFS sorting = Delis-Kaplan executive function system sorting test; JLO = judgment of line orientation test; COWAT = controlled oral word association test; NPI = Neuropsychiatric personality inventory.

*Due to non-normality median and inter quartile range are provided.*
orientation test (JLO; see also Table 1). On the NPI, patients scored higher on agitation, depression, anxiety, apathy, irritability and disinhibition compared to HCs.

**Brain volumes, thalamus volume and thalamic lesions**

Normalized GM volume was not different between patients and HCs ($p = 0.180$), but patients had less WM volume compared to HCs ($p = 0.001$, see Table 1). Thalamus volume was lower in MS patients ($p < 0.001$), which correlated with all cognitive tests except for the JLO and COWAT. In MS, whole brain WM-LL ranged from 0.45–69.86 mL, with a median of 9.74 mL. Lesions in the thalamus were rare (38 patients with no lesions, 22 patients with one lesion, seven patients with two lesions and six patients with three lesions).

**Volumes of the thalamic nuclear groups and integrity measures of the associated tracts**

In Table 2, the measures of the thalamic nuclear groups are presented. The normalized volumes (mL) of the anterior, medial and lateral thalamic portions were similar for MS patients compared to HCs. The volume of the posterior thalamic part was lower in MS patients compared to HCs ($p = 0.008$).

The mean FA in the NAWM of the tract emanating from the anterior part was decreased in patients with MS compared to controls ($p = 0.015$), while no differences could be observed in the mean FA in the medial, lateral and posterior tracts of the thalamus.

Patients had a higher MD compared to HCs in the anterior ($p = 0.021$) and posterior ($p = 0.021$) tracts from the thalamus. No differences were seen in the MD of the lateral and medial tracts.

**Thalamic tract lesion volume, integrity and cognitive performance**

Weighted LL within all tracts was negatively correlated with all cognitive tests (except for JLO) in MS, see also Table 3.

FA within the lateral and medial tracts was not associated with any of the cognitive measures. However, FA within the anterior tract was positively correlated with tests of verbal learning and memory (CVLT, $\rho = 0.38$), visuospatial memory (BVMT-R, $\rho = 0.55$), information processing speed (SDMT, $\rho = 0.59$, PASAT, $\rho = 0.43$) and executive functioning (D-KEFS. $\rho = 0.36$). No association was found between FA in the anterior tract and verbal fluency (COWAT), nor between FA and visuospatial judgment (JLO). In the posterior tract, FA was positively correlated with verbal learning and memory ($\rho = 0.33$), visuospatial memory ($\rho = 0.40$), information processing speed (SDMT, $\rho = 0.45$, PASAT, $\rho = 0.34$) and visuospatial
Thalamic tracts, cognition and disinhibition in MS

**Table 2.** Thalamic measures in patients with MS compared to healthy controls.

<table>
<thead>
<tr>
<th></th>
<th>MS (n = 73)</th>
<th></th>
<th>HC (n = 18)</th>
<th></th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median</td>
<td>IQR</td>
<td>Median</td>
<td>IQR</td>
<td></td>
</tr>
<tr>
<td>Normalized thalamic volume mL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medial</td>
<td>4.61</td>
<td>4.34–5.03</td>
<td>4.74</td>
<td>4.42–4.99</td>
<td>0.451</td>
</tr>
<tr>
<td>Posterior</td>
<td>5.09</td>
<td>4.58–5.50</td>
<td>5.34</td>
<td>5.14–5.77</td>
<td>0.008</td>
</tr>
<tr>
<td>Lateral</td>
<td>5.57</td>
<td>5.14–6.03</td>
<td>5.83</td>
<td>5.18–6.43</td>
<td>0.090</td>
</tr>
<tr>
<td>Anterior</td>
<td>0.84</td>
<td>0.74–0.97</td>
<td>0.80</td>
<td>0.65–0.89</td>
<td>0.271</td>
</tr>
<tr>
<td>DTI metrics within tracts</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FA - Medial</td>
<td>0.46</td>
<td>0.44–0.47</td>
<td>0.47</td>
<td>0.45–0.48</td>
<td>0.753</td>
</tr>
<tr>
<td>FA - Posterior</td>
<td>0.44</td>
<td>0.43–0.47</td>
<td>0.47</td>
<td>0.45–0.48</td>
<td>0.058</td>
</tr>
<tr>
<td>FA - Lateral</td>
<td>0.47</td>
<td>0.43–0.47</td>
<td>0.47</td>
<td>0.46–0.48</td>
<td>0.753</td>
</tr>
<tr>
<td>FA - Anterior</td>
<td>0.42</td>
<td>0.39–0.44</td>
<td>0.45</td>
<td>0.43–0.46</td>
<td>0.015</td>
</tr>
<tr>
<td>MD - Medial (x10–3)</td>
<td>0.839</td>
<td>0.812–0.877</td>
<td>0.813</td>
<td>0.801–0.841</td>
<td>0.073</td>
</tr>
<tr>
<td>MD - Posterior (x10–3)</td>
<td>0.845</td>
<td>0.812–0.884</td>
<td>0.812</td>
<td>0.787–0.832</td>
<td>0.021</td>
</tr>
<tr>
<td>MD - Lateral (x10–3)</td>
<td>0.809</td>
<td>0.783–0.843</td>
<td>0.795</td>
<td>0.779–0.813</td>
<td>0.073</td>
</tr>
<tr>
<td>MD - Anterior (x10–3)</td>
<td>0.886</td>
<td>0.859–0.927</td>
<td>0.861</td>
<td>0.844–0.881</td>
<td>0.021</td>
</tr>
<tr>
<td>Weighted lesion volume within tracts</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medial</td>
<td>37.34</td>
<td>12.06–107.56</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Posterior</td>
<td>91.18</td>
<td>10.74–358.78</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lateral</td>
<td>111.20</td>
<td>29.87–418.12</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anterior</td>
<td>20.12</td>
<td>6.30–52.15</td>
<td>-</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: MS = multiple sclerosis; HC = healthy controls; DTI = diffusion tensor imaging; FA = fractional anisotropy; MD = mean diffusivity.

judgement ($\rho = 0.37$). No relations were found between FA in the posterior tract and executive functioning and between FA and verbal fluency.

Tract MD in all four tracts, was negatively correlated with visuospatial memory, information processing speed (PASAT, SDMT), verbal learning and memory and executive functioning. The MD in the lateral tract was also negatively correlated with the COWAT (verbal fluency). An association with visuospatial judgment (JLO) was absent in all four tracts. See also Supplementary Material e-results.

No correlations were found between volumes of the four different thalamic parts and any of the cognitive measures.
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Thalamic tract lesion volume, integrity and neuropsychiatry

Weighted LL within all four tracts correlated positively with disinhibition and agitation (see Table 3). Additionally, disinhibition positively correlated with MD in all the tracts emanating from the thalamus, while it was negatively correlated to FA in the anterior thalamic tract solely ($\rho = -0.39$). Euphoria and agitation positively correlated with MD in the lateral and medial tracts and euphoria was negatively correlated to FA in the later thalamic tract.

The most important imaging predictor for cognition and neuropsychiatry

Multivariate regression analysis was performed including the following whole brain imaging parameters: WM volume, GM volume, total T2 LL and the more specific thalamic parameters: thalamic volume, mean thalamic tract FA, mean thalamic tract MD and mean thalamic tract LL. Additionally, years of education and sex were included in the model. Multivariate analysis showed that 48.1% of the variance in cognitive performance in MS ($F = 22.63, p < 0.001$) could be explained by whole brain T2 LL (standardized $\beta = -0.40, p = 0.001$), thalamic tract MD (standardized $\beta = -0.32, p = 0.006$) and years of education (standardized $\beta = 0.19, p = 0.03$).

Euphoria could be explained by mean thalamic tract FA, however, the explained variance was limited to adjusted $R^2 = 0.085$ and therefore not of importance. Agitation was explained for 12.2% by thalamic volume ($F = 10.68, \text{standardized } \beta = -0.37 \text{ } p = 0.002$) while mean thalamic tract LL explained 18.8% of the variance in disinhibition ($F = 17.16, \text{standardized } \beta = 0.446, p < 0.001$).

Discussion

We studied cognitive impairment and neuropsychiatric symptoms in MS patients by focusing on the anterior, posterior, lateral and medial thalamic nuclear groups and their associated WM tracts.

Worse cognitive performance in MS patients was related to increased lesion load and increased MD within all four tracts originating from the thalamic subdivisions. On the contrary, FA changes in patients were only detected within the tracts of the anterior and posterior parts of the thalamus, while no such correlations were detected in the lateral and medial tracts.

Specifically, FA was related to executive functioning (DKEFS) solely in the anterior tract, while an association between FA and a task for visuospatial judgment (JLO) was only detected in the posterior tract of the thalamus. From an anatomical point of view, this can be well understood since dense connections run from the anterior thalamic nuclei to the prefrontal cortex, which
Thalamic tracts, cognition and disinhibition in MS

Venouso spatial judgment on the other hand, is mainly mediated via the occipito-parietal areas of the brain (30) and is therefore associated with the posterior thalamic tract. In a previous study, both FA and MD changes were found in the anterior tract of the thalamus related to cognitive functioning.

Table 3. Thalamic tract fractional anisotropy and mean diffusivity measures and the correlations with cognition and neuropsychiatry in patients with MS.

<table>
<thead>
<tr>
<th>Cognition</th>
<th>Neuropsychiatry</th>
</tr>
</thead>
<tbody>
<tr>
<td>CVLT-T</td>
<td>BVMT</td>
</tr>
<tr>
<td>FA&lt;sub&gt;MED&lt;/sub&gt;</td>
<td>ns</td>
</tr>
<tr>
<td>FA&lt;sub&gt;POST&lt;/sub&gt;</td>
<td>0.334*</td>
</tr>
<tr>
<td>FA&lt;sub&gt;LAT&lt;/sub&gt;</td>
<td>ns</td>
</tr>
<tr>
<td>FA&lt;sub&gt;ANT&lt;/sub&gt;</td>
<td>0.384**</td>
</tr>
<tr>
<td>MD&lt;sub&gt;MED&lt;/sub&gt;</td>
<td>−0.442**</td>
</tr>
<tr>
<td>MD&lt;sub&gt;POST&lt;/sub&gt;</td>
<td>−0.543**</td>
</tr>
<tr>
<td>MD&lt;sub&gt;LAT&lt;/sub&gt;</td>
<td>−0.490**</td>
</tr>
<tr>
<td>MD&lt;sub&gt;ANT&lt;/sub&gt;</td>
<td>−0.417**</td>
</tr>
<tr>
<td>WLV&lt;sub&gt;MED&lt;/sub&gt;</td>
<td>−0.460**</td>
</tr>
<tr>
<td>WLV&lt;sub&gt;POST&lt;/sub&gt;</td>
<td>−0.515**</td>
</tr>
<tr>
<td>WLV&lt;sub&gt;LAT&lt;/sub&gt;</td>
<td>−0.519**</td>
</tr>
<tr>
<td>WLV&lt;sub&gt;ANT&lt;/sub&gt;</td>
<td>−0.468**</td>
</tr>
</tbody>
</table>

Spearman correlations, Bonferroni corrected; *p ≤ 0.05, **p ≤ 0.01

Abbreviations: ANT = Anterior part of the thalamus; LAT = Lateral part of the thalamus; MED = Medial part of the thalamus; POST = Posterior part of the thalamus; FA = fractional anisotropy; MD = mean diffusivity; WLV = weighted lesion volume within tract; CVLT-T = California Verbal Learning Test Total learning; BVMT-R = Brief Visuospatial Memory Test Total learning; SDMT = Symbol Digit Modalities Test; PASAT = Paced Auditory Serial Addition Test; DKEFS = Delis-Kaplan Executive Function System; JLO = Judgment of Line Orientation; COWAT = Controlled Oral Word Association Test; ns = not significant.
in benign MS patients, which is similar to our finding. However, in that study information regarding the other thalamic tracts is lacking (31).

Important players for the prediction of overall cognitive functioning in MS are whole brain T2 lesion load and average thalamic tract MD, which are both equally significant. Previously, the importance of cortico-thalamic tracts in explaining cognitive functioning in MS has been shown (32). However, in this study damage of specific thalamic tracts explained cognitive functioning above all other MRI parameters. In our study, whole brain T2 lesion load explained part of the variance in cognition, probably due to a difference in patient population (i.e., our cohort had a longer disease duration and included also patients with secondary-progressive MS).

Studies on neuropsychiatric symptoms in MS and the role of thalamic pathology are scarce. In our study, agitation, euphoria and disinhibition were associated with thalamic tract changes. Increased lesion volume and increased MD in all four thalamic pathways were associated with more disinhibition and more agitation. Again, changes in FA were rather specific and related to disinhibition only in the anterior tract. Changes in the WM connections between the orbitofrontal cortex and the anterior part of the thalamus are most likely explaining the relationship with disinhibited behavior (33). Damage to thalamic tracts (average thalamic tract lesion load) was the main and only imaging predictor for disinhibition in MS and explained approximately 20% of the variance, whereas thalamic volume was the main predictor for agitation. Previously, brain atrophy was described as a predictor of disinhibition in MS (34). Here thalamic tract lesion load was the only neuroimaging predictor (GM and WM volume did not survive the regression analysis), which suggests that damage to the thalamic tracts might be more specific for disinhibition.

Euphoria was associated with increased MD in the medial and lateral tracts of the thalamus and decreased FA in the lateral thalamic tract. Yet, in the regression model, none of the imaging measures were of significant importance to predict euphoria, suggesting that other factors are of more influence on this particular neuropsychiatric symptom.

Unfortunately, the resolution of our DTI measures forced us to reduce the number of thalamic nuclei to four subdivisions, which is less regionally specific than the anatomical subdivisions from histopathological studies. Visual inspection assured an anatomically correct segmentation. We did not detect differences in the four volumes of the independent thalamic nuclei (except in the posterior part). This might be best explained by a relatively large variance of the measured volumes of the individual nuclei as a result of heterogeneity (disease duration, disease severity) and a sample size that may be too small to account for it. The volume of all thalamic nuclei combined, resulted in smaller thalamic volumes in the
patient group ($p = 0.05$). No differences in GM volume could be detected between patients and controls. When subdividing the patients into RRMS and SPMS patients, the SPMS patients had significant GM volume loss compared to healthy controls, which is in line with the existing literature (35). Due to the relatively few SPMS patients no significant differences could be detected between patients and controls.

In conclusion:

- Lesion load and mean diffusivity within the anterior, posterior, medial and lateral thalamic tracts are associated with cognition, disinhibition and agitation;
- Changes in fractional anisotropy were related to cognition and disinhibition only in the anterior and posterior thalamic tracts;
- Whole brain T2 lesion load and mean diffusivity within the thalamic tracts are equally important in predicting cognitive functioning;
- Lesion load within the thalamic tracts is the main predictor for disinhibited behavior;
- Discriminating between the different nuclei of the thalamus and their associated tracts, and more specifically the anterior and posterior thalamic tracts, allows us to explain cognitive functioning and neuropsychiatric symptoms in MS.
Chapter 4.2

References


Supplementary materials

**E-method: Pipeline of constructing the atlas**

1. The construction of the atlas involved running bedpostx (part of FSL) for each of the 27 HCs to estimate the voxelwise diffusion parameter distributions and probabilistic tractography (FSL probtrackx, 5000 streamlines per voxel) using the predefined thalamic sections as seed regions.

2. For each of the 27 HCs, the probabilistic maps of the tracts were then binarized using a 0.25% threshold of the total number of seeded streamlines, non-linearly registered to MNI152 space, and averaged to construct a single probabilistic map for each tract in standard space. This resulted in eight probabilistic maps (left/right, 4 thalamic seed-regions) with voxelwise values between zero and one, which formed the atlas of the tracts associated with the thalamic sections.

3. To obtain a subject-specific segmentation, the probabilistic atlas was finally propagated to the individual space of both the patients and HCs part of the study cohort, using non-linear registration and linear interpolation.

4. For each subject and tract separately, the weighted average FA and the weighted average MD values inside the normal-appearing white matter (NAWM) of each tract, as well as the lesion load (LL) within the tract, was computed using the atlas probability values as a weighting factor. Values within the center of each tract have a higher certainty that the area accurately belonged to that particular tract compared to values located at the border of the tract. Using weighted scores therefore emphasized all values within the center of the tract. In order to only include NAWM, the weighting factors of voxels belonging to GM, CSF or lesions were set to zero using the earlier derived SIENAX and lesion segmentations.

5. The weighted LL inside each tract was computed as described above. Note that the weighted LL is a relative, instead of an absolute value, and cannot be compared with normalized (unweighted) whole brain lesion volumes (see Supplementary Figure 1).

**E-results**

The total learning score of both the CVLT and BVMT gives a good reflection of verbal and spatial memory respectively. To reduce the number of comparisons we therefore decided not to add the delayed scores to the model. However, similar correlations with DTI metrics for
the delayed recall scores compared to the total learning scores were found. MD in the anterior tract correlated for −0.405 with the CVLT delayed recall and for −0.381 with the BVMT delayed recall. FA in the anterior tract correlated for 0.432 with CVLT delayed recall and for 0.451 with BVMT delayed recall. All correlations reached statistical significance at $p \leq 0.001$. 