Widespread white matter connectivity abnormalities in narcolepsy type 1: A diffusion tensor imaging study

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A B S T R A C T

Narcolepsy type 1 is caused by a selective loss of hypothalamic hypocretin-producing neurons, resulting in severely disturbed sleep-wake control and cataplexy. Hypocretin-producing neurons project widely throughout the brain, influencing different neural networks. We assessed the extent of microstructural white matter organization and brain-wide structural connectivity abnormalities in a homogeneous group of twelve drug-free patients with narcolepsy type 1 and eleven matched healthy controls using diffusion tensor imaging with multimodal analysis techniques. First, tract-based spatial statistics (TBSS) was carried out using fractional anisotropy (FA) and mean, axial and radial diffusivity (MD, AD, RD). Second, quantitative analyses of mean FA, MD, AD and RD were conducted in predefined regions-of-interest, including sleep-wake regulation-related, limbic and reward system areas. Third, we performed hypothalamus-seeded tractography towards the thalamus, amygdala and midbrain. TBSS analyses yielded brain-wide significantly lower FA and higher RD in patients. Localized significantly lower FA and higher RD in the left ventral diencephalon and lower AD in the midbrain, were seen in patients. Lower FA was also found in patients in left hypothalamic fibers connecting with the midbrain. No significant MD and AD differences nor a correlation with disease duration were found. The brain-wide, localized ventral diencephalon (comprising the hypothalamus and different sleep- and motor-related nuclei) and hypothalamic connectivity differences clearly show a heretofore underestimated direct and/or indirect effect of hypocretin deficiency on microstructural white matter composition, presumably resulting from a combination of lower axonal density, lower myelination and/or greater axon diameter.

1. Introduction

Narcolepsy type 1 is a severely disabling neurological condition, characterised by excessive daytime sleepiness (EDS), attention deficits, cataplexy, sleep paralysis, hypnagogic hallucinations and disturbed nocturnal sleep. It has been suggested that it is an autoimmune-mediated disorder but the exact pathophysiology remains to be discovered (Liblau et al., 2015). Patients with narcolepsy type 1 show a selective loss of hypothalamic hypocretin neurons producing hypocretin (or orexin) (Peyron et al., 2000; Thannickal et al., 2000). In an average healthy brain, each hemisphere comprises roughly 50,000–80,000 hypocretin-producing neurons, localized exclusively within the lateral and posterior hypothalamus (Moore et al., 2001). Their axonal projections diffusely disperse over the brain excluding the cerebellum with particularly dense projections to the locus coeruleus, ventral tegmental area and the tuberomammillary, raphe, laterodorsal tegmental and pedunculopontine tegmental nuclei (Sakurai, 2007). Hypocretin affects various neural networks; it maintains sleep-wake state stability, energy homeostasis, reward system regulation and cognitive and mood control (Nishino and Sakurai, 2005). Given the variety of complaints in narcolepsy, a pertinent question is to what extent local and global white brain matter integrity and connectivity are affected.

DTI is an MRI technique enabling in-vivo modelling of microstructural white matter (WM) morphology by means of water diffusivity variations in different tissue types (Basser et al., 1994). Scalar variables of fractional anisotropy (FA) and mean, axial and radial diffusivity (MD,
AD, RD) are normally used as outcome measures. Fractional anisotropy (FA), reflects how directionally constrained the diffusion of water is along axons. While higher FA values might indicate more coherent, intact axons and/or higher myelination, lower FA may imply loss of WM integrity and/or injury. MD is composed of the mean diffusion in all three diffusion directions as a quantitative measure of alterations in the extracellular volume. AD captures the most prominent direction, parallel to axon orientation and RD the mean of the remaining perpendicular two directions. Lower AD reflects axonal damage, whereas higher RD is related to lower axonal density, lower myelination and/or greater axon diameter (Chanraud et al., 2010; Soares et al., 2013; Song et al., 2002). The outcome measures reflect the sum of different underlying tissue characteristics within one voxel. Possible differences are often explained by a combination of aforementioned morphological features.

Six diffusion tensor imaging (DTI) studies investigating structural WM morphology in people with narcolepsy compared to controls have previously been reported (Juvodden et al., 2018; Menzler et al., 2012; Nakamura et al., 2013; Park et al., 2016; Scherfler et al., 2012; Tezer et al., 2018). Findings are inconsistent, but disruptions were found in the hypothalamus–thalamus–orbitofrontal pathway and the brainstem as parts of the sleep-wake regulation system, as well as in the reward and limbic system and the corticospinal tract. The findings of previous DTI studies including patients with narcolepsy type 1 or narcolepsy with cataplexy are summarized in Supplementary Table I. Inconsistencies likely result from methodological differences between studies, including the use of central nervous system stimulants (Alicata et al., 2009), small groups, age variability (Giorgio et al., 2010), differences in patient characteristics (narcolepsy related to H1N1-vaccination, disease duration and severity) and restricted outcome measures (only FA and MD), making the interpretation complex.

Previous studies solely used whole-brain tract-based spatial statistics (TBSS) or whole-brain voxel-based morphometry (VBM) with diffusion metrics, which both are unable to assess interregional connectivity and compare regions subject to anatomical variation. Our aim was to identify the extent and nature of microstructural WM integrity disruptions in people with narcolepsy type 1 using a three-way analysis strategy, combining whole-brain TBSS (I), region-of-interest (ROI) analyses (II) and tractography (III). Where TBSS identifies whole-brain microstructural WM differences within regions of considerable proportion, the ROI-based analyses also assess average microstructural WM integrity in regions with anatomical variability, while hypothalamus-seeded tractography specifically assesses the hypothalamic connectivity throughout the brain. Given the diversity in symptoms experienced by patients, we hypothesized that patients in comparison to controls in general show brain-wide lower FA, higher RD and unaffected MD (I), localized lower FA in symptom-related regions (e.g. ventral diencephalon, midbrain, thalamus) (II) and in hypothalamus-seeded tracts to the thalamus, amygdala and midbrain. (III). In the TBSS analyses (I) we also expected significant relationships between longer disease durations, higher sleepiness scores and lower FA in regions related to the arousal system, including the pons, midbrain, thalamus and prefrontal cortex.

2. Materials and methods

2.1. Participants

Twelve adults with narcolepsy type 1 were recruited at our outpatient narcolepsy clinic (Sleep-Wake Centre SEIN) and eleven age and sex group-matched healthy controls were recruited through adverts in local newspapers. To be included, all subjects had to be 18–65 years old; right-handed according to the Edinburgh Handedness Scale (Oldfield, 1971) and have normal or corrected-to-normal vision. People with narcolepsy type 1 were diagnosed according to the 3rd edition of the International Classification of Sleep Disorders (American Academy of Sleep Medicine, 2014) and had to be treatment-naïve or off medication for ≥14 days prior to the study. Exclusion criteria consisted of current use of psychotropic drugs; current diagnosis of any other serious medical conditions; contraindications for MRI studies and macroscopic structural brain abnormalities (tumor, ventricle enlargement, cortical atrophy or vascular lesions).

In line with diagnostic criteria, the three patients without clear-cut cataplexy had hypocretin-1 levels determined in their cerebrospinal fluid (CSF). One patient had a concentration slightly higher (138 pg/mL) than the according to international standards assessed 110 pg/mL diagnostic threshold for narcolepsy type 1 (Mignot et al., 2002). This patient was still included because of the typical clinical phenotype and the still deficient hypocretin-1 level. All demographic and DTI analyses were repeated excluding this subject to verify that this person was no outlier. In total, CSF hypocretin-1 levels were available in nine patients as part of regular clinical care, of which all were hypocretin deficient. All patients had positive HLA-typing for DQB1*0602. Seven patients were drug naïve, and five discontinued their stimulants (4× methylphenidate and 1× modafinil) at least 2 weeks prior to study start. The five subjects had been using medication for 1–5 months prior to discontinuation.

Following subject screening to assess study eligibility, written informed consent was obtained from all subjects. The study protocol was approved by the Medical Ethical Committee of LUMC.

2.2. Data acquisition

All subjects completed a survey on general characteristics (e.g. age and previous medication use). In addition, the Dutch Adult Reading Test (Schmand et al., 1991) was completed to assess intelligence (IQ) and the Epworth Sleepiness Scale (ESS) ( Johns, 1991) was administered as a measure of daytime sleepiness. All data collection was performed during the afternoon and subjects were asked to refrain from caffeine containing substances for at least 24 h prior to examination.

MRI data were acquired with a high-field 3 T Philips Achieva MRI scanner (Philips Healthcare, Best, the Netherlands) and a 32-channel head coil with sponge cushions to minimize head movements. Three-dimensional T1-weighted images (220 slices; TR 8.2 ms; TE 3.8 ms; inversion time 670.4 ms; FOV 240 × 240 × 220 mm; matrix size 240 × 240; flip angle 8°; 1 × 1 × 1 mm3 voxel size) and single-shot, gradient-echo, echo-planar imaging (EPI) sequences for DTI (60 slices; TR = 6700 ms; TE = 72 ms; FOV 224 × 224 × 120 mm; matrix size 112 × 112; flip angle = 90°; 2 × 2 × 2 mm3 voxel size) were obtained. DTI was carried out twice with reversed k-space readout along 46 non-collinear directions with b = 1000 s/mm2 and one unweighted b = 0 s/mm2 image, each. Acquisition time was 6.2 min for T1-weighted scans and 5.9 min for each DTI scan.

One experienced reviewer (JKG), blinded to the scan identity, inspected the unprocessed T1-weighted and DTI scans and processing steps as mentioned hereafter. Subcortical segmentations, cortical parcellations and the registration of ROIs to DTI subject space were additionally manually corrected if needed.

2.3. T1-weighted image processing

After visual examination of the T1-weighted images for artefacts and macroscopic brain abnormalities, the intensity was normalized and Freesurfer’s MRI analysis software package (v5.3.0 Developmental) was used to automatically generate subcortical segmentations and cortical parcellations (Fischl et al., 2002; Iglesias et al., 2015).

ROIs were determined a priori on the basis of previously reported neuroimaging results (Supplementary Table I) and included regions likely related to narcolepsy complaints. All regions were part of the sleep-wake regulation, limbic or reward system and consisted of the hypothalamus, ventral diencephalon, thalamus, midbrain, amygdala, parahippocampal, anterior cingulate (merged rostral and caudal
anterior cingulate WM), medial and lateral orbitofrontal WM.

The hypothalamus was manually segmented in both hemispheres using standardized criteria (Nieuwenhuys et al., 1988). The anterior boundary of the hypothalamus consisted of the first coronal slice posterior of where the anterior commissure became discontinuous. Posteriorly, the last slice was where the mammillary bodies (included) diverted one another in the mid-sagittal plane. The hypothalamus inferiorly ended where the optic chiasm and infundibular stalk began and the anterior–posterior commissure plane was used for the superior delineation, which in itself was not included. Laterally, the segmentation was limited by WM of the internal capsule. Hypothalamus segmentation was blindly performed, twice for every subject. Corresponding maps were visually compared and combined to form the eventual segmentation.

2.4. Diffusion tensor image processing

After automatic brain extraction (Jenkinson et al., 2005), DTI scans were corrected for susceptibility-, eddy current- and subject movement-induced distortions with the FMRIB Software Library’s (FSL, v5.0) topup and eddy tools (Andersson et al., 2003; Andersson and Sotiropoulos, 2016). Calculated off-resonance field maps were applied and ‘dropout-slices’ were replaced using non-parametric, Gaussian process modelled predictions (Andersson et al., 2016; Andersson and Sotiropoulos, 2016).

All ROIs were subsequently resampled to the corresponding pre-processed b0 images using linear coregistration of the T1-weighted image, to precisely identify subject-unique ROIs for local and tractography analyses. Incomplete imaging of an ROI due to incorrect field-of-view alignment resulted in the exclusion of this subject from the analyses including this ROI. The posterior-to-anterior-encoded DTI scan of one control subject was missing and therefore only the anterior-to-posterior-encoded images were used for this subject.

2.4.1. Tract-based spatial statistics (I)

In FSL’s TBSS processing stream (Smith et al., 2006), the two DTI scans were averaged and projected onto an alignment-invariant skeleton image and thresholded at a > 0.2 FA level. All individual FA, MD, AD and RD maps were subsequently projected onto the skeleton, smoothed (5 mm) and fed into permutation-based voxelwise cross-subject statistics with 10,000 permutations and threshold-free cluster enhancement to identify between-group differences. The FA results were masked with FSL’s JHU white matter tractography atlas’ tracts (Hua et al., 2008) and presented as absolute and relative significance p-values, which was higher in patients [10.08 ± 3.00 vs. 2.64 ± 1.96; t(21) = −6.97, p < .001]. The patient with a 1.87 pg/mL hypocretin-1 level was not on any of the demographic or DTI analyses.

3. Results

3.1. Demographic and clinical data

Data from age-and-sex-matched patients and controls were included in a 12:11 ratio. As shown in Table 1, there were no significant demographic and clinical differences between the groups, except for ESS score, which was higher in patients [10.08 ± 3.00 vs. 2.64 ± 1.96; t(21) = −6.97, p < .001]. The patient with a 1.87 pg/mL hypocretin-1 level was not on any of the demographic or DTI analyses.

3.2. Tract-based spatial statistics (I)

Whole-brain microstructural WM differences were observed in people with narcolepsy in comparison to controls by means of overall significantly lower FA and higher RD (Fig. 1). Most prominent differences were found in the bilateral ventral diencephalon (comprising i.e. hypothalamus), thalamus, midbrain, pons, (orbito-) frontal, anterior cingulate, primary motor and somatosensory WM, internal capsule and the corpus callosum. No differences between groups were seen in other contrasts and in the cerebellum altogether. No significant relationships between time since EDS onset and ESS score and FA were present. Randomly creating two new groups pooling evenly cases and controls to detect spurious results did not yield significant differences.

Masking the FA results with the JHU white matter tractography atlas’ tracts showed highly significant clusters with lower FA widely spread throughout the brain (Fig. 2); up to 68.1% of the left corticospinal tract voxels were significantly different. In contrast, only the parahippocampal cingulum WM showed a limited number of significantly different voxels [left: 2.1%, right: 3.3%].
3.3. Region-of-interest analyses (II)

As expected from the TBSS results, statistically significant lower FA was seen in patients (Table 2, Fig. 3) in the ventral diencephalon (left: $F_{(1,19)} = 16.024, p = .001$; right: $F_{(1,19)} = 6.604, p = .019$), left thalamus $F_{(1,19)} = 5.365, p = .032$ and midbrain $F_{(1,19)} = 4.394, p = .050$. Patients also showed higher MD in the left ventral diencephalon $F_{(1,19)} = 5.511, p = .050$. Patients' tracts connecting the left hypothalamus with the midbrain showed lower FA (Table 3) significantly lower AD and higher RD in the midbrain were found (I) which found brain-wide signifi cantly lower FA and in different MD in drug-naïve patients with narcolepsy type 1, including the corpus callosum, midbrain and bilateral cingulate gyrus, thalamus, internal capsule. Higher RD was also reported in the midbrain and bilateral thalamus and lower AD in the corpus callosum. Discrepancies with this study in AD and RD differences most likely result from their different microstructural WM disruptions we found (I), are generally in keeping with a previous large-scale TBSS study (Juvodden et al., 2018) which reported signifi cantly lower FA in patients with narcolepsy. A fourth TBSS study (Menzler et al., 2012) reported both lower and higher FA in the right hypothalamus, brainstem, corticospinal tract and temporal, frontal and parietal WM. Inconsistencies with this latter study in FA disruption directions are possibly related to the use of psycho tropic drugs (mostly central nervous system stimulants) in these patients compared to our drug-free population, as medication is known to influence WM morphology (Alicata et al., 2009).

Our results are in partial agreement with the whole-brain VBM-style study by Scherfler et al. (2012) that reported significantly lower FA and hypocretin deficiency on microstructural white matter composition in patients with narcolepsy type 1.

4. Discussion

Microstructural WM properties were significantly abnormal in people with narcolepsy type 1 in the three methodologies used. Whole-brain lower FA and higher RD – excluding the cerebellum – were seen in people with narcolepsy type 1 within functional networks such as the sleep-wake regulation, limbic and reward system and corticospinal tract (Fig. 4). In patients mean lower FA and higher RD in the left ventral diencephalon and lower AD in the midbrain were found. Patients' tracts connecting the left hypothalamus with the midbrain showed lower FA (II). This study suggests a heretofore underestimated modulatory effect

Table 1

| Characteristics of the study population. | Patients (n = 12) | Controls (n = 11) | P-value |
|----------------------------------------|------------------|------------------|--------|
| Male:female (N:N)                      | 8:4              | 7:4              | 0.879  |
| Age (years)                            |                  |                  |        |
| Mean (SD)                              | 33.25 (10.50)    | 31.82 (13.39)    | 0.777  |
| Median (IQR)                           | 32 (24.25–40.5)  | 26 (23–38.5)     |        |
| Range                                  | 21–52            | 18–55            |        |
| IQ score (mean, SD)                    | 110.58 (10.73)   | 111.30 (8.25)    | 0.865  |
| Age of onset EDS (years, mean, SD)     | 19.42 (9.15)     |                  |        |
| EDS duration (years, median, IQR)      | 10.00 (6.00–25.25)|                  |        |
| Cataplexy presence                     | 9/12             |                  |        |
| Cataplexy and/or hypocretin deficient* | 12/12            |                  |        |
| HLA DQB1*0602 presence                 |                  |                  |        |
| Sleep latency (minutes, mean, SD)      | 6.26 (3.64)      |                  |        |
| SOREM periods (mean, SD)               | 2.58 (1.57)      |                  |        |
| Polysomnography                        |                  |                  |        |
| TST (minutes, mean, SD)                | 426.50 (25.33)   |                  |        |
| Sleep efficiency (% of TST, mean, SD)  | 91.89 (5.49)     |                  |        |
| Stage 1 sleep (% of TST, mean, SD)     | 12.75 (7.19)     |                  |        |
| Stage 2 sleep (% of TST, mean, SD)     | 43.91 (10.38)    |                  |        |
| Stage 3–4 sleep (% of TST, mean, SD)   | 17.40 (8.65)     |                  |        |
| Stage REM sleep (% of TST, mean, SD)   | 23.86 (6.00)     |                  |        |
| PLMI (mean, SD)                        | 4.84 (7.50)      |                  |        |
| EDS, excessive daytime sleepiness; ESS, Epworth sleepiness scale; HLA, human leukocyte antigen; MSLT, multi sleep latency test; PLMI, periodic leg movement index; SOREM, sleep onset rapid eye movement; TST, total sleep time.
| * One patient had a deficient hypocretin-1 level (138 pg/mL) slightly above the diagnostic threshold (< 110 pg/mL). This patient was still included given the clinically typical narcolepsy and still deficient hypocretin-1 level.

4.1. Tract-based spatial statistics (I)

The widespread microstructural WM disruptions we found (I), are generally in keeping with a previous large-scale TBSS study (Juvodden et al., 2018) which found brain-wide significantly lower FA and different MD in drug-naïve patients with narcolepsy type 1, including the corpus callosum, midbrain and bilateral cingulate gyrus, thalamus, internal capsule. Higher RD was also reported in the midbrain and bilateral thalamus and lower AD in the corpus callosum. Discrepancies with this study in AD and RD differences most likely result from their different lower disease duration (5.7 years) and the almost exclusive inclusion of patients that developed narcolepsy type 1 after H1N1-vaccination. Similar results were also seen in two other TBSS studies (Park et al., 2016; Tezer et al., 2018) which reported selective extensively lower FA in patients with narcolepsy. Significant areas included the corpus callosum (the genu for Park et al., 2016 and body for Tezer et al., 2018), anterior internal capsule and thalamus. Tezer et al. (2018) additionally found lower FA in the midbrain, temporal lobe and cerebellum, whereas Park et al. (2016) also reported differences in the bilateral anterior cingulate, frontal lobe and left posterior internal capsule and thalamus. These regions were also found to be abnormal in our study, except for the cerebellum. This slight difference could be related to the absence of hypocretin measurements in these two studies and the lower b-values that were used for DTI acquisition, which are known to influence the diffusion metrics.

A fourth TBSS study (Menzler et al., 2012) reported both lower and higher FA in the right hypothalamus, brainstem, corticospinal tract and temporal, frontal and parietal WM. Inconsistencies with this latter study in FA disruption directions are possibly related to the use of psychotropic drugs (mostly central nervous system stimulants) in these patients compared to our drug-free population, as medication is known to influence WM morphology (Alicata et al., 2009).
higher MD in the orbitofrontal cortex, anterior cingulate, singular lower MD in the ventral tegmental area, dorsal raphe nuclei and hypothalamus and lower FA measurements in the inferior frontal and inferior temporal cortices. Comparability with this study is difficult though, as included subjects on average were over 55 years of age and generally still used psychotropic medication. A second VBM study (Nakamura et al., 2013) has described increased MD in the left inferior frontal gyrus and left parahippocampal gyrus/amygdala and lower MD in the postcentral gyrus in narcolepsy with cataplexy patients compared to controls. Interestingly, the left laterization of amygdala differences that was reported by Nakamura et al. (2013) could not be replicated in this study as we found no significant differences in FA and MD in the left
amygdala in the ROI analyses. The reliability of these VBM-style approaches remains problematic given its susceptibility to bias from incorrect subject alignment and the inconsistent use of smoothing, making interpretability complex (Bookstein, 2001; Jones et al., 2005; Van Hecke et al., 2010). This could have especially affected the latter study due to its limited spatial resolution (3.5 mm voxel thickness) and the 12 mm smoothing that was used.

4.2. Region-of-interest analyses (II)

In the ROI analyses, lower FA was found in patients, especially in the highly significant left ventral diencephalon. In the post-hoc analyses, significantly higher RD was also found in this region in addition to significantly lower AD in the midbrain. (II). The ventral diencephalon is filled with a variety of sleep-wake regulation areas, including those in the hypothalamus and superior midbrain. Other included regions are related to motor functioning and the reward system, such as the subthalamic nucleus, substantia nigra and red nucleus and connecting fibers of the crus cerebri, lenticular fasciculus and medial

![Fig. 2. The averaged FA map masked with the JHU tractography atlas labels (red-yellow). Reported as absolute significantly different voxel count within the label/total voxels within the label, corresponding percentage of significant voxels, minimum p-value found within the label.](image)
The lemniscus. The lower AD in the midbrain indicates axonal loss in patients (Wheeler-Kingshott and Cercignani, 2009) and could be explained by the loss of hypothalamic projections running through the midbrain towards sleep-wake-, motor- and reward-regulating nuclei in the brainstem, including the ventral tegmental area, locus coeruleus and laterodorsal tegmental, raphe and pedunculopontine tegmental nuclei (Sakurai, 2007). The ROI findings further validate the correspondence between WM integrity disruptions in patients with narcolepsy and sleep-wake, reward system and motor complaints.

### 4.3. Tractography analyses (III)

Although not always significant, the overall lower FA and generally lower volume of patients’ tracts indicate a correlation between hypocretin deficiency and WM integrity disruptions in fiber bundles connecting with the hypothalamus, especially passing through the

| Region-of-interest FA and MD analyses. | Fractional anisotropy | Mean diffusivity |
|----------------------------------------|-----------------------|------------------|
|                                       | Patients (mean, SD)   | Controls (mean, SD) | P-value | Patients (mean, SD) | Controls (mean, SD) | P-value |
| Left thalamus                          | 0.370 (0.015)         | 0.387 (0.020)     | 0.032   | 0.755 (0.020)       | 0.745 (0.019)       | 0.229   |
| Right thalamus                         | 0.348 (0.013)         | 0.360 (0.015)     | 0.061   | 0.785 (0.018)       | 0.788 (0.029)       | 0.746   |
| Left amygdala                          | 0.245 (0.017)         | 0.244 (0.027)     | 0.942   | 0.811 (0.017)       | 0.807 (0.027)       | 0.614   |
| Right amygdala                         | 0.240 (0.017)         | 0.238 (0.013)     | 0.897   | 0.838 (0.021)       | 0.848 (0.040)       | 0.499   |
| Left ventral diencephalon              | 0.489 (0.016)         | 0.521 (0.021)     | 0.001   | 0.846 (0.061)       | 0.799 (0.042)       | 0.030   |
| Right ventral diencephalon             | 0.482 (0.017)         | 0.506 (0.028)     | 0.019   | 0.866 (0.043)       | 0.834 (0.056)       | 0.129   |
| Left hypothalamus                      | 0.293 (0.026)         | 0.299 (0.026)     | 0.483   | 1.144 (0.127)       | 1.328 (0.399)       | 0.165   |
| Right hypothalamus                     | 0.280 (0.021)         | 0.292 (0.026)     | 0.186   | 1.210 (0.128)       | 1.209 (0.209)       | 0.968   |
| Left lateral orbitofrontal WM          | 0.393 (0.020)         | 0.401 (0.020)     | 0.301   | 0.751 (0.025)       | 0.741 (0.017)       | 0.287   |
| Right lateral orbitofrontal WM         | 0.397 (0.021)         | 0.401 (0.018)     | 0.588   | 0.740 (0.029)       | 0.731 (0.022)       | 0.430   |
| Left medial orbitofrontal WM           | 0.368 (0.024)         | 0.376 (0.030)     | 0.359   | 0.750 (0.032)       | 0.743 (0.020)       | 0.612   |
| Right medial orbitofrontal WM          | 0.385 (0.028)         | 0.397 (0.033)     | 0.368   | 0.767 (0.020)       | 0.770 (0.026)       | 0.738   |
| Left anterior cingulate WM             | 0.535 (0.025)         | 0.534 (0.023)     | 0.921   | 0.768 (0.039)       | 0.769 (0.034)       | 1.000   |
| Right anterior cingulate WM            | 0.555 (0.024)         | 0.566 (0.038)     | 0.365   | 0.803 (0.031)       | 0.814 (0.032)       | 0.457   |
| Left parahippocampal WM                | 0.413 (0.008)         | 0.420 (0.032)     | 0.391   | 0.786 (0.014)       | 0.779 (0.034)       | 0.530   |
| Right parahippocampal WM               | 0.419 (0.018)         | 0.422 (0.028)     | 0.604   | 0.803 (0.027)       | 0.803 (0.035)       | 1.000   |
| Midbrain                               | 0.530 (0.050)         | 0.575 (0.018)     | 0.050   | 1.038 (0.121)       | 1.119 (0.077)       | 0.086   |

WM, white matter.

* Unrounded p-value = .049693.
midbrain (III). No direct comparison of these results could be made as this is the first tractography study in people with narcolepsy. Our re-
ported overall projection pattern is in line with previous hypothalamic tractography results (Lemaire et al., 2011), where dense projections were found in healthy subjects to the thalamus, amygdala, para-
hippocampus and brainstem and cortically widespread bundles mainly to the central gyri, frontal and occipital lobe. Our study found dense tracts connecting the hypothalamus with the thalamus, amygdala and midbrain in all subjects, whereas more diffuse connections were often seen to the orbitofrontal and parahippocampal WM. Tracts towards the anterior cingulate WM were less prevalent. Connections to other, not mentioned regions were out of this study’s scope. The reason why more diffuse tracts are sometimes not detected when using tractography, is related to the partial volume e-
deficiency. Involvement of a secondary mechanism or the presence of a cascade of indirect e-
deficiency as this could also give insight in the pathophysiology of different symptoms experienced by patients. It could also explain the susceptibility of patients to develop certain symptoms.

The absence a significant correlation between time since symptom onset and FA was unexpected. Similar results were previously seen (Juvodden et al., 2018; Park et al., 2016) and might be due to the re-
lation of end-stage narcolepsy in all studies. This indicated that when the chronic narcolepsy state has been reached, the brain reaches structural end-stage white matter composition. A comparison between patients in different disease stages, implementing a multimodal analysis strategy, should be the target of future research.

4.5. Methodological strengths and limitations

We assessed a homogeneous group of patients using a multimodal analysis strategy, to improve data quality and interpretability. All controls were age- and sex-matched and a standardized data acquisition and a three-way analysis strategy were used. By also adding RD and AD to the outcome measures, this study fills an important gap in the current literature as a more in-depth interpretation of the origin of the differences is included.

The relatively sporadic significance in the ROI-based (II) and tracto-
graphy analyses (III) – compared to the widely present TBSS ab-
normalities – could mainly be attributed to intrinsic methodological differences, as averaging outcome measures within and between ROIs omits significant subregions, while TBSS (I) identifies subter per-voxel differences (Mukherjee et al., 2008). The effect of crossing fibers and the relatively low proportion of hypocretin-producing neurons within the fiber bundles they follow, could further explain this. However, TBSS (I) is incapable of providing information on interregional connectivity and regions subject to anatomical variability as only regions containing dense white matter bundles are included (Bach et al., 2014). This could also explain the combination of significantly lower AD in patients in the midbrain in the ROI-based analyses (II) and the absence of significant AD differences in the TBSS results (I). Given the distinct strengths and limitations of the individual methods and as previously suggested by Wakana et al. (2007), we would recommend standard implementation of combined analysis techniques for future research.

Although findings are robust, some limitations of our study also need to be addressed. An important limitation to the presented study is the limited sample size that was included. Future research should

Table 3
Post-hoc region-of-interest AD and RD analyses.

|                  | Patients (mean, SD) | Controls (mean, SD) | P-value | Patients (mean, SD) | Controls (mean, SD) | P-value |
|------------------|---------------------|---------------------|---------|---------------------|---------------------|---------|
| **Axial diffusivity** |                     |                     |         |                     |                     |         |
| Left thalamus    | 1.061 (0.023)       | 1.063 (0.013)       | 0.766   | 0.602 (0.021)       | 0.587 (0.025)       | 0.106   |
| Left ventral diencephalon | 1.330 (0.090)   | 1.298 (0.047)       | 0.225   | 0.604 (0.049)       | 0.550 (0.043)       | 0.008   |
| Right ventral diencephalon | 1.357 (0.079) | 1.330 (0.056)       | 0.307   | 0.621 (0.035)       | 0.586 (0.060)       | 0.097   |
| Midbrain         | 1.735 (0.183)       | 1.924 (0.701)       | 0.004   | 0.690 (0.012)       | 0.717 (0.010)       | 0.614   |
| **Radial diffusivity** |                     |                     |         |                     |                     |         |

Table 4
Hypothalamic white matter connectivity.

Fractional anisotropy

|                  | Patients | Controls | P-value |
|------------------|----------|----------|---------|
| Left – left thalamus | 0.411 (0.033) | 0.426 (0.022) | 0.229 |
| Right – right thalamus | 0.412 (0.038) | 0.430 (0.027) | 0.191 |
| Left – left amygdala | 0.426 (0.050) | 0.438 (0.045) | 0.319 |
| Right – right amygdala | 0.399 (0.053) | 0.418 (0.044) | 0.239 |
| Left – midbrain | 0.419 (0.031) | 0.458 (0.030) | 0.007 |
| Right - midbrain | 0.405 (0.035) | 0.428 (0.041) | 0.130 |

Mean diffusivity

|                  | Patients | Controls | P-value |
|------------------|----------|----------|---------|
| Left thalamus    | 0.900 (0.085)       | 0.842 (0.075)       | 0.127   |
| Left ventral diencephalon | 0.918 (0.078)   | 0.911 (0.074)       | 0.814   |
| Right ventral diencephalon | 0.846 (0.115) | 0.796 (0.058)       | 0.222   |
| Midbrain         | 0.868 (0.134)       | 0.861 (0.062)       | 0.845   |

Tract volume

|                  | Patients | Controls | P-value |
|------------------|----------|----------|---------|
| Left thalamus    | 1.909 (0.753)       | 1.897 (0.760)       | 0.991   |
| Right – right thalamus | 2.017 (1.148) | 2.052 (1.044)       | 0.745   |
| Left – left amygdala | 0.773 (0.324) | 0.799 (0.392)       | 0.850   |
| Right – right amygdala | 0.782 (0.355) | 0.873 (0.461)       | 0.560   |
| Left – midbrain | 2.156 (0.912)       | 2.894 (0.890)       | 0.034   |
| Right - midbrain | 2.395 (1.540)       | 3.595 (1.468)       | 0.079   |

Each row represents the tracts connecting the left or right hypothalamus with the mentioned ROI. Values are reported as mean (SD). * Tract volume is in cm³.
include larger samples to enable reliable subgroup analyses investigating the relationship between the presence of narcolepsy-related symptoms, neuropsychological measures, and the observed microstructural white matter abnormalities of patients with narcolepsy. Additionally, five patients had used psychotropic medication before inclusion. Even though medication was discontinued, this could have influenced results.

It should also be noted that the ventral diencephalon is a more reliable ROI than the hypothalamus for the ROI analyses, as this last region is particularly susceptible to CSF contamination and hence to partial volume artefacts. This is less relevant for tractography analyses, as a result of the used FA threshold.

5. Conclusion

The reported whole-brain (I), localized ROI (II) and connectivity (III) differences clearly show lower FA in patients with narcolepsy compared to controls. In combination with the TBSS results showing brain-wide higher RD, this would indicate that narcolepsy type 1 patients suffer from a combination of lower axonal density, lower myelination and/or greater axon diameter (Song et al., 2002; Wheeler-Kingshott and Cercignani, 2009). Given the extent of the WM abnormalities, our results indicate a more complex cascade or a secondary pathway causing these differences, not solely explained by hypocretin deficiency on its own.

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Declaration of Competing Interest

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