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Assessment of glymphatic function in narcolepsy using DTI-ALPS index

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ABSTRACT

Introduction: Sleep is a modulator of glymphatic activity which is altered in various sleep disorders. Narcolepsy is a sleep disorder characterized by excessive daytime sleepiness (EDS), rapid onset of rapid eye movement (REM) sleep, cataplexy, disturbed night sleep with fragmentation. It is categorized into two types, type 1 (NT1) and type 2 (NT2) depending on the presence of cataplexy and/or absence of orexin. We sought for alterations in glymphatic activity in narcoleptic patients using diffusion tensor imaging (DTI) along perivascular space (ALPS) index on magnetic resonance imaging (MRI).

Material and methods: Adult patients diagnosed with NT1 or NT2 who had polysomnography (PSG) and MRI with DTI were included in the study. Sleep recording included Epworth Sleepiness Scale (ESS) score, sleep latency during multiple sleep latency test (MSLT), sleep efficiency during night PSG, wake after sleep onset (WASO), REM sleep latency during PSG, percentage of non-REM (NREM), REM sleep and wakefulness during night PSG. DTI-ALPS index was calculated for each patient and age-sex matched healthy control(HC)s.

Results: The study group was composed of 25 patients [F/M = 15/10, median age = 34 (29.5–44.5)], 14 with NT1 and 11 with NT2 disease. ESS, WASO and percentage of wakefulness were significantly higher in NT1 patients ($p < 0.05$). Mean DTI-ALPS was not significantly different neither between narcoleptic patients and HCs, nor between NT1 and NT2 patients (all, $p > 0.05$). However, DTI-ALPS was negatively correlated with WASO ($r = -0.745$, $p = 0.013$) and percentage of wakefulness ($r = -0.837$, $p = 0.005$) in NT1 patients. DTI-ALPS correlated negatively with percentage of N1 sleep ($r = -0.781$, $p = 0.005$) but positively with REM percentage ($r = 0.618$, $p = 0.043$) in NT2 patients.

Conclusion: In this study, DTI-ALPS was not significantly different in narcoleptic patients than the HCs. However, the glymphatic index as assessed by DTI-ALPS correlated with PSG parameters; negatively with WASO, percentage of wakefulness in NT1, percentage of N1 sleep in NT2, and positively with REM sleep in NT2. A tendency for a reduction in DTI-ALPS in NT1 patients compared to both NT2 patients and HCs was also found. These findings might show the first evidence of an alteration of glymphatic activity, especially in NT1 patients, thus warrant further prospective studies in larger size of narcoleptic patient cohorts.

1. Introduction

Maintaining homeostasis through waste clearance is essential for sustaining functional activity in all biological tissues. Devoid of classical lymphatic vessels, brain waste clearance had been a mystery for a long time. However, in recent years, the discovery of the brain wide glia-dependent waste clearance system, i.e. glymphatic system and the meningeal lymphatics let us gain new insights for the maintenance of brain homeostasis [1–3]. Glymphatic activity is mainly composed of three steps: 1) Glymphatic influx of cerebrospinal fluid (CSF) into the brain parenchyma through the arterial perivascular space with an aquaporin 4 (Aqp4) dependent manner, 2) CSF mixing with interstitial fluid (IF), carrying solutes and macromolecules towards perivenous perivascular space, 3) glymphatic efflux of mixed CSF-IF, carrying solutes to the venous

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perivascular space in an Aqp4 dependent manner. CSF is drained by either perineural pathways or meningeal lymphatic vessels [4]. Amyloid beta (Aß) protein and tau oligomers are also carried from IF to CSF via this system [1], in advance, several studies confirmed impaired glymphatic function in neurological-neurodegenerative disorders [5], particularly Alzheimer's disease (AD) and Parkinson disease (PD) [6,7].

Another novel study demonstrated that sleep and anesthesia had a triggering effect on glymphatic activity [8]. Glymphatic influx was drastically (95%) decreased in awake state compared to sleep and anesthesia in mice. Also, Aß clearance showed a twofold increase in sleep and anesthesia compared to awake state [8]. Further studies confirmed that one night sleep deprivation or slow wave sleep (SWS) disruption is associated with higher CSF levels of Aß in humans [9,10]. These findings were also supported by positron emission tomography (PET) and magnetic resonance imaging (MRI). After one night sleep deprivation, Shokri-Kojori et al. [11] demonstrated 5% increase of Aß burden in the brain parenchyma of healthy individuals with PET imaging and Eide et al. [12] showed delayed metabolic clearance from brain parenchyma using MRI with intrathecal administration of contrast material. Since then, human MRI studies investigating the relationship of sleep and glymphatic function have been increasing. As intrathecal administration of contrast agent is an invasive way to asses glymphatic activity, a diffusivity index, called diffusion tensor imaging (DTI) – along perivascular space (ALPS), was suggested by Taoka T et al., in 2017 [13]. This index correlates with the diffusivity along perivascular space in the periventricular region at the level of lateral ventricles' bodies, therefore glymphatic activity. Studies using the DTI-ALPS index have confirmed that DTI-ALPS index was decreased in patients with AD, PD and idiopathic normal pressure hydrocephalus, in correlation with reduced glymphatic activity in these diseases [13–16]. Besides, recent studies also confirmed altered glymphatic activity in patients with obstructive sleep apnea and rapid eye movement (REM) sleep behavior disorder by using DTI-ALPS index [17–20].

In this study, we sought to determine whether any alterations of glymphatic activity is present in patients with narcolepsy using DTI-ALPS index. Narcolepsy is a chronic sleep-wake cycle disorder, characterized with rapid onset of REM sleep status and excessive daytime sleepiness (EDS) with difficulty in sustaining awakeness. The disease has two forms, narcolepsy type 1 (NT1) and type 2 (NT2) [21]. NT1 is more common and pathophysiologically better understood with loss of orexin (hypocretin) neurons in lateral hypothalamus. The difference between NT1 and NT2 in terms of diagnostic criteria is the presence of cataplexy and/or CSF orexin levels equal to or lower than 110 pg/ml [21]. On the other hand, the pathophysiology of NT2 has not been fully understood, yet. Although characterized by the same symptoms of NT1, CSF orexin levels are normal and cataplexy is absent in NT2. Several studies investigated narcoleptic patients with DTI and revealed widespread microstructural alterations in the brain compared to healthy controls [22–30]. But, no study assessed glymphatic activity using DTI-ALPS index in narcoleptic patients, yet.

Disturbed nocturnal sleep (DNS) with sleep fragmentation is a major electrophysiologically feature common to both types of narcolepsy. In addition to that, mean sleep latency is lower than 8 min in multiple sleep latency test (MSLT) and at least two sleep-onset REM sleep periods are recorded (SOREMPs) in polysomnography (PSG) and/or MSLT findings of both NT1-NT2. However, there are no clear electrophysiological diagnostic criteria to distinguish the two subtypes. But clinically, REM sleep behavior disorder (RBD) is frequently seen with narcolepsy, particularly in NT1 [31]and the presence of REM sleep without atonia (RSWA) is suggested to be a sensitive and specific parameter for distinguishing NT1 from NT2 [32,33]. Accordingly, we hypothesized that DNS, sleep fragmentation and the presence of RBD/RSWA affecting the sleep quality might alter glymphatic activity in these patients. Furthermore, there might be a difference in glymphatic activity between NT1 and NT2 patients related to the presence of RBD/RSWA. To test this hypothesis, we used the DTI-ALPS index and PSG findings using the previously collected data of narcoleptic patients [27].

2. Material and Methods

The institutional review board (IRB) approved the study (GO, 22/309). Patients with narcolepsy and the healthy subjects who underwent MRI including DTI for a previous study [27] constituted the study group. Because all subjects had given informed consent previously, informed consent was waived for the current study by our IRB.

2.1. Patient selection

All patients who were diagnosed with narcolepsy after an evaluation in our PSG laboratory between 2014 and 2016 and who had DTI MRI for the previous study [27] were retrospectively evaluated for further participation in this study. The diagnosis of NT1 and NT2 was made according to the International Classification of Sleep Disorders, 3rd edition [21]. However, for both groups CSF orexin levels were unavailable.

The inclusion criteria of the patients for this study were set as having: 1) clinically and electrophysiologically confirmed diagnosis of narcolepsy, either NT1 or NT2, according to the International Classification of Sleep Disorders [21]. 2) MR imaging including DTI at the beginning of the diagnosis of narcolepsy (drug naive patients, i.e. patients not taking any REM sleep-suppressing medications including tricyclic antidepressants, SSRIs, SNRIs, stimulants, sodium oxybate or sedating drugs), 3) Normal physical and neurological examination, 4) No medical record of systemic and/or neurological disorder. Patients were excluded if they had 1) apnea-hypopnea index $\geq$5 h in PSG, 2) any structural lesion on MRI (including small vessel disease).

Sex- and age-matched HCs served as the control group in the previous study [27] also constituted the control group in the present study. The inclusion criteria for the present study were set as: 1) MR imaging including DTI, 2) No sign of sleep disorder in the questionnaire forms including sleep apnea scale of the sleep disorders questionnaire, Epworth Sleepiness Scale (ESS), medical outcome study-sleep scale (MOS-SS), Pittsburgh Sleep Scale (PSS), 3) Completely normal physical and neurological examination and 4) No medical record of systemic and/or neurological disorder. HCs were excluded from the study if any of the following was present: 1) any structural lesion on MRI (including small vessel disease), 2) subjects taking any REM sleep-suppressing or sedating drugs.

For this aim, the following information was retrieved from the medical records of all patients and HCs: Demographic information including sex and age (at data collection and at symptom onset), physical and neurological examination (at data collection), history of systemic and/or neurologic disorders including hypertension, diabetes mellitus, neuroinflammatory and neurovascular disorders, medication at the time of the diagnosis including REM sleep-suppressing or sedating drugs. Previous MR images were also re-evaluated for the presence of any intracranial lesion suggesting tumors, neuroinflammatory or neuroimmunologic disorders or neurovascular conditions.

2.2. Polysomnography and sleep evaluation

All patients and HCs had complete questionnaire forms including sleep apnea scale of the sleep disorders questionnaire, ESS, MOS-SS, PSS. Each patient was monitored for two days in the video-EEG-PSG monitoring unit using a 32-channel EEG system (Grass-Telefactor, XLTEK). Scalp electrodes were placed according to the standard 10–20 system. The other parameters recorded included electrooculogram (EOG), submental electromyogram and electrocardiogram (ECG), respiratory effort and airflow, oxyhemoglobin saturation, and anterior tibialis EMG. Second night-sleep studies were manually scored in all patients for sleep stages in 30-s epochs with an expanded EEG montage by an experienced neurophysiologist. Sleep was scored according to the revised American Academy of Sleep Medicine (AASM) criteria [31]. Overnight PSG was immediately followed by MSLT for all patients [34,35].

So, for patients ESS score, sleep latency during MSLT, sleep efficacy during night PSG, wake after sleep onset (WASO), REM sleep latency during PSG, percentage of non-REM (NREM) sleep stages, and REM sleep during night PSG were retrospectively collected from medical records. Loss of REM atonia was also identified according to AASM, as an excessive amount of sustained or intermittent loss of REM atonia and/or excessive phasic muscle twitch activity of the submental and/or limb EMGs during REM sleep. The presence of RSWA and/or RBD were noted according to video-PSG recordings-reports of all patients. Due to lack of a well-established method for RSWA quantification, RSWA measurements were not obtained.

2.3. MRI acquisition and image processing

All patients and healthy controls (HCs) had MR imaging performed on a 3T MRI scanner (Ingenia, philips) equipped with an eight-channel phase-array head coil.

All patients had MRI in a month after the diagnosis of narcolepsy, before the initiation of therapy. Imaging protocol was set as follows: Fluid attenuated inversion recovery (FLAIR) imaging (TR/TE/TI: 4800/355/1650 ms, FOV: 240, matrix: 352 × 352, slice thickness: 2 mm, pixel size: 1.03 × 1.04 mm^2); DTI of the whole brain, single-shot EPI; TR/TE:3051.2/92.8 ms, b value of 800 s/mm2, 32 independent diffusion weighted directions and one b0 acquisition, FOV: 233 mm, matrix: 176 × 176, 50 slice with 2.5-mm thickness without intersection gap, pixel size:1.32 × 1.32 mm2).

For the correction of susceptibility induced artifacts in DTI images, first the structural FLAIR images and non-diffusion weighted (b0) images were utilized by the Synb0-DISCO tool to estimate undistorted b0 images and off-resonance field coefficients [36]. The estimated field coefficients were then input to the FSL TOPUP tool to correct diffusion-weighted images using the Jacobian interpolation method for all subjects [37,38]. To suppress the background regions and non-brain tissue voxels, FSL BET tool was used to generate a binary brain mask [39]. Diffusion tensor fitting was performed with the FSL DTIFIT tool. Fractional Anisotropy (FA) map was overlaid with the primary eigenvector to generate color FA map to be used for region of interest (ROI) selection [40,41]. Two neuroradiologists with 5 and 20 years of experience, draw square-shaped ROIs spanning 3 × 3 pixels in consensus on the slice at the level of lateral ventricle for both projection and association fibers and checked with JHU-DTI White Matter Atlas provided by FSL. Left hemisphere was used as all participants were right-handed. We were also ensured the ROIs were not contaminated with other structures by checking multiple diffusion directions. DTI-ALPS index was calculated using a custom script in MATLAB as follows [13]:

$$DTI-ALPS = mean(D_{x,proj}, D_{x,assoc}) \,/\, mean(D_{y,proj}, D_{z,assoc})$$

Here, $D_{x,proj}$ and $D_{x,assoc}$ represent diffusivities along x-axis for projection and association fiber ROIs, respectively. In addition, $D_{y,proj}$ and $D_{z,assoc}$ represent the diffusivity along y-axis for projection fiber ROI and the diffusivity along z-axis for association fiber ROI, respectively. (see Fig. 1).

2.4. Statistical analysis

Statistical analysis was performed with the SPSS 25.0 package program. Depending on the type of variables (continuous vs. categorical) and the distribution of the data, comparison tests was performed using one-way ANOVA or Kruskal-Wallis test for multiple groups, Chi-squared test, independent Student's t-test or Mann Whitney-U tests were used for two group comparisons. Correlation analysis was performed using the Spearman's rank correlation to reveal the relationship between DTI-ALPS index and PSG findings. The level of significance was 0.05 in all tests.

3. Results

The PSG data and MR images of the patients (n = 28) collected for the previous study were retrospectively reviewed. One patient had obstructive sleep apnea, one patient had previous brain surgery, and one patient had distorted MR images, therefore excluded. The study group was composed of 25 patients [F/M = 15/10, median age = 34 (29.5–44.5)]. Fourteen patients with cataplexy attacks constituted NT1 patients [F/M = 9/5, median age = 33.5 (27.5–38.5)]. NT2 group was composed of 11 patients [F/M = 6/5, median age = 40 [30–47]]. Hypocretin/orexin levels of cerebrospinal fluid were unavailable. There were 11 age and sex matched HCs [F/M = 6/5, median age = 36 (31–58)] (Table 1). The median ages were not statistically different between groups. PSG findings of each group were summarized in Table 1. ESS, WASO and the percentage of wakefulness were significantly higher in NT1 patients. The ratio of RBD and/or RSWA was 85% in NT1 and 81% in NT2. There was no statistically significant difference between subgroups in terms of the presence of RBD and RSWA (p = 0.531).

Mean DTI-ALPS of narcoleptic patients and HCs were 1.699 ± 0.046 and 1.814 ± 0.086, respectively and were not significantly different (p = 0.211). There was no correlation between age and DTI-ALPS in patients with narcolepsy. Mean DTI-ALPS of NT1 and NT2 patients were 1.631 ± 0.049 and 1.786 ± 0.08, respectively. Although NT1 patients had a tendency to have lower DTI-ALPS index compared to NT2 and HCs, no statistical difference was observed between NT1 and NT2 narcolepsy patients and HCs (p = 0.140) (Table 1). There was no significant difference in mean DTI-ALPS values when patients were grouped according to the presence of RBD and RSWA (p = 0.235).

DTI-ALPS was negatively correlated with WASO (r = −0.5, p = 0.029), percentage of wakefulness (r = −0.501, p = 0.029) and percentage of N1 sleep (r = −0.678, p = 0.001) in all patients. There was no statistically significant correlation between DTI-ALPS and duration of disease, total sleep time, percentages of N2 and N3 sleep across the patients. Correlation analysis was reapplied to the subgroups of the disease. DTI-ALPS was negatively correlated with WASO (r = −0.745, p = 0.013) and percentage of wakefulness (r = −0.837, p = 0.005) in NT1 patients. In addition, DTI-ALPS index showed a mild positive correlation with N3%, but it did not reach statistical significance (r = 0.552, p = 0.078). On the other hand, DTI-ALPS correlated negatively with percentage of N1 sleep (r = −0.781, p = 0.005) but positively with REM percentage (r = 0.618, p = 0.043) in NT2 patients. These findings are summarized in Table 2.

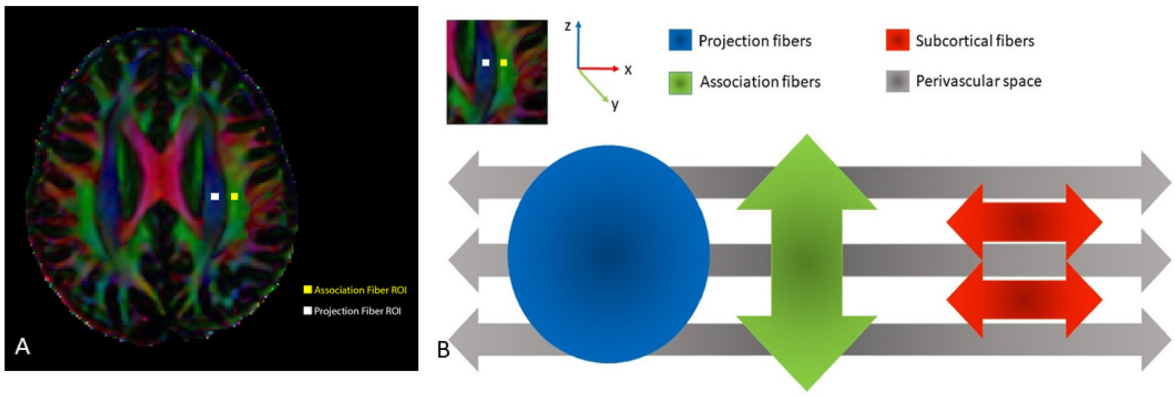

Fig. 1. (A) The diffusion tensor was fitted for each subject and the FA map was overlaid with the primary eigenvector to create a color FA map. Square-shaped 3 × 3 ROIs were marked on the left projection (white) and association fibers (yellow) at the level of the lateral ventricle. (B) Schematic illustration of the orientation of the fibers and perivascular space in the periventricular area. Note that direction of the perivascular space is perpendicular to the directions of projection and association fibers.

Table 1
Summary of findings.

	Type 1 Narcolepsy (n = 14)	Type 2 Narcolepsy (n = 11)	Healthy Controls (n = 11)	*p*
Demographic information				
Age	33.5 (27.5–38.5)	40 [30–47]	36 (31–58)	0.436
Sex (F/M)	9/5	6/5	6/5	0.846
Polysomnographic Findings				
Epworth Sleepiness score	20.4 ± 2.87	16.73 ± 4.24		0.016*
Total sleep duration (minutes)	426.2 ± 50.85	455.36 ± 30.61		0.137
WASO	71.5 ± 29.08	37.78 ± 25.98		0.017*
N1 (%)	8.09 ± 1.92	8 ± 4.45		0.951
N2 (%)	47.18 ± 11.43	53.45 ± 9.29		0.174
N3 (%)	14.27 ± 6.24	12.91 ± 4.18		0.555
REM (%)	16.18 ± 4.24	16.82 ± 3.25		0.697
Wakefulness (%)	16.22 ± 6.22	10 ± 5.63		0.035*
Presence of RBD/RSWA (n)	12	9		0.531
DTI-ALPS index				
DTI-ALPS	1.631 ± 0.049	1.786 ± 0.08	1.814 ± 0.086	0.140

*represents significant difference ($p < 0.05$).

4. Discussion

This study revealed some important findings showing the relation between narcolepsy and glymphatic function of the brain. First, we observed a tendency of reduced DTI-ALPS index, as an imaging marker of glymphatic activity in patients with cataplexy (NT1) compared to those without cataplexy (NT2) and HCs, albeit not reaching statistical significance. In addition, DTI-ALPS index showed negative correlations with WASO and percentage of wakefulness in the NT1 group. In the NT2 group, this activity correlated negatively with N1%, but positively with REM percentage. Based on these findings, increased wakefulness with fragmentation of sleep are associated with altered glymphatic function in narcoleptic patients and this alteration is possibly more prominent in NT1 patients compared to NT2.

Although not reaching statistical significance, the decrease in DTI-ALPS index of NT1 patients in our study is the first trace that glymphatic activity could be impaired also in narcolepsy as well as other sleep disorders [17–20]. Increased glymphatic activity during normal sleep is thought to be driven by the oscillations during SWS, i.e. N3 stage of sleep, and interstitial volume increase with the inhibition of noradrenergic system [8]. Importance of the integrity of SWS for glymphatic activity was confirmed with several studies. SWS disruption is strongly and significantly correlated with CSF Aß levels [42], while partial sleep deprivation preserving SWS resulted in no accumulation of proteins in CSF as shown in a study by Olsson M et al. [43]. A study using different anesthetic regimens further showed importance of the power of slow wave oscillations and its correlation with glymphatic influx [44]. In the established form of narcolepsy, DNS with sleep fragmentation, frequent shifts between sleep stages and arousals become prominent, which causes increased N1 stage of sleep. Although both our patient groups have good total sleep time, N3 stage was reduced (<20% of total amount of sleep) in both compared to normal sleep [45]. NT1 patients had significantly increased percentages of wakefulness and ESS

Table 2
Correlation of DTI-ALPS index with PSG findings.

	WASO	Wakefulness %	N1%	N2%	N3%	REM %
$DTI\text{-}ALPS_{pt}$						
r	−0.500*	−0.501*	−0.678*	0.344	0.190	0.335
p	0.029	0.029	0.001	0.117	0.397	0.128
$DTI\text{-}ALPS_{NT1}$						
r	−0.745*	−0.837*	−0.353	0.118	0.552	0.032
p	0.013	0.005	0.287	0.729	0.078	0.925
$DTI\text{-}ALPS_{NT2}$						
R	−0.25	−0.17	−0.781*	0.388	−0.051	0.618
p	0.516	0.638	0.005	0.238	0.883	0.043

$DTI\text{-}ALPS_{pt}$: represents all narcoleptic patients.
$DTI\text{-}ALPS_{NT1}$: represents patients with NT1.
$DTI\text{-}ALPS_{NT2}$: represents patients with NT2.
r: Spearmann's correlation coefficient.
p: statistically significant <0.05.

compared to NT2 patients, suggesting lower sleep quality and integrity. All these might contribute to the mildly lower DTI-ALPS levels in NT1 patients compared to NT2 patients. Additionally, DTI-ALPS was negatively correlated with percentage of wakefulness and WASO in NT1 and percentage of N1 sleep in NT2. Unfortunately, due to lack of PSG studies in our HCs and limited number of patients, the relationship between DTI-ALPS and N3 sleep percentage could not be assessed, which creates a major limitation in current study. However, a mild positive correlation was found between DTI-ALPS index and N3% in NT1, not reaching statistical significance ($r = 0.552$, $p = 0.078$). Further prospective studies might explain whether a true relation exist between these parameters in narcoleptic patients.

Other sleep related symptoms/signs of narcolepsy include the presence of RBD and RSWA, both of which are also associated with and accepted as prodromal markers of α-synucleinopathies [46]. The glymphatic dysfunction in α-synucleinopathies has already been shown and also, a very recent study revealed impaired glymphatic function in patients with RBD and PD in correlation with the severity of clinical findings [20]. Therefore, RBD/RSWA might impair the glymphatic function, not directly but destroying the sleep structure. There is no definite evidence of NT1 to be an α-synucleinopathy [47], but with aforementioned changes in glymphatic activity in RBD and α-synucleinopathies, we wanted to show whether the presence of RBD/RSWA would affect the DTI-ALPS index. In our small cohort, RBD/RSWA was documented in most of the patients ($n = 21$, 84%) in either type of narcolepsy and the comparison of DTI-ALPS showed no difference. Considering the limited number of patients in our study, larger cohorts are needed to reveal whether RBD/RSWA might have an effect on glymphatic function including patients with α-synucleinopathies and narcolepsy.

Orexin regulates wakefulness and REM sleep and NT1 is characterized by presence of cataplexy and/or deficient orexin in CSF [48]. The neurodegenerative disorders that are related with glymphatic dysfunction like AD and PD, are also shown to have alterations in the orexinergic system. Moderate-severe Alzheimer patients had higher CSF orexin levels [49]. There is loss in orexin neurons in PD in both patients and animal models [50,51]. CSF orexin levels were unavailable in our cohort but, NT1 patients, who are supposed to have deficient orexin, had a tendency to have slightly lower DTI-ALPS compared to NT2 and HCs, not reaching statistical significance. This finding may raise a suspicion on a slightly higher alteration of glymphatic function in NT1 patients. However, our study had a retrospective design with limited number of patients and unavailable CSF orexin levels. Further studies are needed to investigate whether a glymphatic dysfunction is present in narcoleptic patients with larger cohorts including CSF orexin levels.

There are several limitations in our study. First of all, the number of patients was very limited. Orexin levels from CSF were unavailable, and the HCs lacked PSG results. Therefore, we could not clearly discuss the effect of PSG findings including sleep fragmentation and RSWA/RBD. In addition, night-sleep quality of patients and HCs before MRI scan that might affect the function of glymphatic system, was unavailable. These limitations could not be overcome due to retrospective design of the study. However, we believe that our findings add to the literature that glymphatic function disturbance in narcolepsy could be investigated in relation to specific sleep components using DTI-ALPS as an imaging marker. Further studies with larger cohorts including narcolepsy patients with and without RBD/RSWA are needed to reveal the relation between various sleep disorders and glymphatic function.

5. Conclusion

Our study shows the first evidence of an alteration of glymphatic activity in narcolepsy patients, assessed by DTI-ALPS. Glymphatic activity in narcoleptic patients were modulated by some of the PSG parameters. Increase in wakefulness and N1 stage of sleep correlated negatively with glymphatic activity. NT1 patients had a lower sleep quality, as well as a tendency to have lower glymphatic activity, compared to NT2 patients.

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CRediT authorship contribution statement

Ekim Gumeler: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Writing – original draft. **Elif Aygun:** Conceptualization, Data curation, Software, Validation, Writing – review & editing. **F. Irsel Tezer:** Conceptualization, Data curation, Investigation, Methodology, Resources, Supervision, Visualization, Writing – review & editing. **Emine Ulku Saritas:** Conceptualization, Data curation, Software, Validation, Writing – review & editing. **Kader K. Oguz:** Conceptualization, Methodology, Supervision, Validation, Visualization, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no conflict of interest.

References

[1] Iliff JJ, Wang M, Liao Y, Plogg BA, Peng W, Gundersen GA, et al. A paravascular pathway facilitates CSF flow through the brain parenchyma and the clearance of interstitial solutes, including amyloid β. Sci Transl Med 2012 Aug;4(147):147ra111.
[2] Louveau A, Smirnov I, Keyes TJ, Eccles JD, Rouhani SJ, Peske JD, et al. Structural and functional features of central nervous system lymphatic vessels. Nature 2015 Jul;523(7560):337–41.
[3] Aspelund A, Antila S, Proulx ST, Karlsen TV, Karaman S, Detmar M, et al. A dural lymphatic vascular system that drains brain interstitial fluid and macromolecules. J Exp Med 2015 Jun;212(7):991–9.
[4] Proulx ST. Cerebrospinal fluid outflow: a review of the historical and contemporary evidence for arachnoid villi, perineural routes, and dural lymphatics. Cell Mol Life Sci 2021 Mar;78(6):2429–57.
[5] Rasmussen MK, Mestre H, Nedergaard M. The glymphatic pathway in neurological disorders. Lancet Neurol 2018 Nov;17(11):1016–24.
[6] Kyrtsos CR, Baras JS. Modeling the role of the glymphatic pathway and cerebral blood vessel properties in Alzheimer's disease pathogenesis. PLoS One 2015;10(10):e0139574.
[7] Sundaram S, Hughes RL, Peterson E, Müller-Oehring EM, Brontë-Stewart HM, Poston KL, et al. Establishing a framework for neuropathological correlates and glymphatic system functioning in Parkinson's disease. Neurosci Biobehav Rev 2019 Aug;103:305–15.
[8] Xie L, Kang H, Xu Q, Chen MJ, Liao Y, Thiyagarajan M, et al. Sleep drives metabolite clearance from the adult brain. Science 2013 Oct;342(6156):373–7.
[9] Ooms S, Overeem S, Besse K, Rikkert MO, Verbeek M, Claassen JAHR. Effect of 1 night of total sleep deprivation on cerebrospinal fluid β-amyloid 42 in healthy middle-aged men: a randomized clinical trial. JAMA Neurol 2014 Aug;71(8):971–7.
[10] Varga AW, Wohlleber ME, Giménez S, Romero S, Alonso JF, Ducca EL, et al. Reduced slow-wave sleep is associated with high cerebrospinal fluid Aβ42 levels in cognitively normal elderly. Sleep 2016 Nov;39(11):2041–8.
[11] Shokri-Kojori E, Wang G-J, Wiers CE, Demiral SB, Guo M, Kim SW, et al. β-Amyloid accumulation in the human brain after one night of sleep deprivation. Proc Natl Acad Sci U S A 2018 Apr;115(17):4483–8.
[12] Eide PK, Vinje V, Pripp AH, Mardal K-A, Ringstad G. Sleep deprivation impairs molecular clearance from the human brain. Brain 2021 Apr;144(3):863–74.
[13] Taoka T, Masutani Y, Kawai H, Nakane T, Matsuoka K, Yasuno F, et al. Evaluation of glymphatic system activity with the diffusion MR technique: diffusion tensor image analysis along the perivascular space (DTI-ALPS) in Alzheimer's disease cases. Jpn J Radiol 2017;35(4):172–8.
[14] Chen H-L, Chen P-C, Lu C-H, Tsai N-W, Yu C-C, Chou K-H, et al. Associations

among cognitive functions, plasma DNA, and diffusion tensor image along the perivascular space (DTI-ALPS) in patients with Parkinson's disease. Oxid Med Cell Longev 2021:4034509. 2021.
[15] Ma X, Li S, Li C, Wang R, Chen M, Chen H, et al. Diffusion tensor imaging along the perivascular space index in different stages of Parkinson's disease. Front Aging Neurosci 2021;13:773951.
[16] Bae YJ, Choi BS, Kim J-M, Choi J-H, Cho SJ, Kim JH. Altered glymphatic system in idiopathic normal pressure hydrocephalus. Park Relat Disord 2021 Jan;82:56–60.
[17] Lee H-J, Lee DA, Shin KJ, Park KM. Glymphatic system dysfunction in obstructive sleep apnea evidenced by DTI-ALPS. Sleep Med 2022 Jan;89:176–81.
[18] Roy B, Nunez A, Aysola RS, Kang DW, Vacas S, Kumar R. Impaired glymphatic system actions in obstructive sleep apnea adults. Front Neurosci 2022;16:884234.
[19] Lee DA, Lee H-J, Park KM. Glymphatic dysfunction in isolated REM sleep behavior disorder. Acta Neurol Scand 2022 Apr;145(4):464–70.
[20] Si X, Guo T, Wang Z, Fang Y, Gu L, Cao L, et al. Neuroimaging evidence of glymphatic system dysfunction in possible REM sleep behavior disorder and Parkinson's disease. NPJ Park Dis 2022 Apr;8(1):54.
[21] American Academy of Sleep Medicine. The international classification of sleep disorders. third ed. Darien, IL: American Academy of Sleep Medicine; 2014.
[22] Scherfler C, Frauscher B, Schocke M, Nocker M, Gschliesser V, Ehrmann L, et al. White and gray matter abnormalities in narcolepsy with cataplexy. Sleep 2012 Mar;35(3):345–51.
[23] Menzler K, Belke M, Unger MM, Ohletz T, Keil B, Heverhagen JT, et al. DTI reveals hypothalamic and brainstem white matter lesions in patients with idiopathic narcolepsy. Sleep Med 2012 Jun;13(6):736–42.
[24] Nakamura M, Nishida S, Hayashida K, Ueki Y, Dauvilliers Y, Inoue Y. Differences in brain morphological findings between narcolepsy with and without cataplexy. PLoS One 2013;8(11):e81059.
[25] Park YK, Kwon O-H, Joo EY, Kim J-H, Lee JM, Kim ST, et al. White matter alterations in narcolepsy patients with cataplexy: tract-based spatial statistics. J Sleep Res 2016;25(2):181–9.
[26] Juvodden HT, Alnæs D, Lund MJ, Agartz I, Andreassen OA, Dietrichs E, et al. Widespread white matter changes in post-H1N1 patients with narcolepsy type 1 and first-degree relatives. Sleep 2018 Oct;41(10).
[27] Tezer FI, Erdal A, Gumusyayla S, Has AC, Gocmen R, Oguz KK. Differences in diffusion tensor imaging changes between narcolepsy with and without cataplexy. Sleep Med 2018;52:128–33.
[28] Gool JK, Fronczek R, Leemans A, Kies DA, Lammers GJ, Van der Werf YD. Widespread white matter connectivity abnormalities in narcolepsy type 1: a diffusion tensor imaging study. NeuroImage Clin 2019;24:101963.
[29] Park HR, Kim HR, Seong J-K, Joo EY. Localizing deficits in white matter tracts of patients with narcolepsy with cataplexy: tract-specific statistical analysis. Brain Imaging Behav 2020 Oct;14(5):1674–81.
[30] Ni K, Liu Y, Zhu X, Tan H, Zeng Y, Guo Q, et al. Changed cerebral white matter structural network topological characters and its correlation with cognitive behavioral abnormalities in narcolepsy type 1. Nat Sci Sleep 2022;14:165–73.
[31] Berry R, Brooks R, Gamaldo C, Harding S, Lloyd R, Marcus C. The AASM manual for the scoring of sleep and associated events : rules, terminology, and technical specifications. Version 2. American academy of sleep medicine. Darien, IL: American Academy of Sleep Medicine; 2016.
[32] Yon MI, Azman F, Yon ME, Tezer FI. Nocturnal rapid eye movement sleep without atonia can Be a diagnostic parameter in differentiating narcolepsy type 1 from type 2. J Clin Neurophysiol Off Publ Am Electroencephalogr Soc 2021 May;38(3):237–41.
[33] Akyildiz UO, Tezer FI, Koc G, Ismailogullari S, Demir AB, Ak AK, et al. The REM-sleep-related characteristics of narcolepsy: a nation-wide multicenter study in Turkey, the REMCON study. Sleep Med 2022 Jun;94:17–25.
[34] Carskadon MA, Dement WC, Mitler MM, Roth T, Westbrook PR, Keenan S. Guidelines for the multiple sleep latency test (MSLT): a standard measure of sleepiness. Sleep 1986 Dec;9(4):519–24.
[35] Littner MR, Kushida C, Wise M, Davila DG, Morgenthaler T, Lee-Chiong T, et al. Practice parameters for clinical use of the multiple sleep latency test and the maintenance of wakefulness test. Sleep 2005 Jan;28(1):113–21.
[36] Schilling KG, Blaber J, Huo Y, Newton A, Hansen C, Nath V, et al. Synthesized b0 for diffusion distortion correction (Synb0-DisCo). Magn Reson Imaging 2019 Dec;64:62–70.
[37] Andersson JLR, Skare S, Ashburner J. How to correct susceptibility distortions in spin-echo echo-planar images: application to diffusion tensor imaging. Neuroimage 2003 Oct;20(2):870–88.
[38] Smith SM, Jenkinson M, Woolrich MW, Beckmann CF, Behrens TEJ, Johansen-Berg H, et al. Advances in functional and structural MR image analysis and implementation as FSL. Neuroimage 2004;23(Suppl 1):S208–19.
[39] Smith SM. Fast robust automated brain extraction. Hum Brain Mapp 2002 Nov;17(3):143–55.
[40] Smith SM, Jenkinson M, Johansen-Berg H, Rueckert D, Nichols TE, Mackay CE, et al. Tract-based spatial statistics: voxelwise analysis of multi-subject diffusion data. Neuroimage 2006;31(4):1487–505.
[41] McCarthy P. FSLeyes (1.4.0). p. Zenodo. https://doi.org/10.5281/zenodo.6511596.
[42] Ju Y-ES, Ooms SJ, Sutphen C, Macauley SL, Zangrilli MA, Jerome G, et al. Slow wave sleep disruption increases cerebrospinal fluid amyloid-β levels. Brain 2017 Aug;140(8):2104–11.
[43] Olsson M, Ärlig J, Hedner J, Blennow K, Zetterberg H. Sleep deprivation and cerebrospinal fluid biomarkers for Alzheimer's disease. Sleep 2018 May;41(5).
[44] Hablitz LM, Vinitsky HS, Sun Q, Stæger FF, Sigurdsson B, Mortensen KN, et al. Increased glymphatic influx is correlated with high EEG delta power and low heart rate in mice under anesthesia. Sci Adv 2019 Feb;5(2):eaav5447.
[45] Carskadon MA, Dement WC. Normal human sleep: an overview. In: Kreiger M, Roth T, Dement WC, editors. Principles and practice of sleep medicine. sixth ed. sixth ed. Philedelphia, PA: Elsevier; 2016.
[46] Dauvilliers Y, Schenck CH, Postuma RB, Iranzo A, Luppi P-H, Plazzi G, et al. REM sleep behaviour disorder. Nat Rev Dis Prim 2018 Aug;4(1):19.
[47] Antelmi E, Pizza F, Donadio V, Filardi M, Sosero YL, Incensi A, et al. Biomarkers for REM sleep behavior disorder in idiopathic and narcoleptic patients. Ann Clin Transl Neurol 2019 Sep;6(9):1872–6.
[48] Kang J-E, Lim MM, Bateman RJ, Lee JJ, Smyth LP, Cirrito JR, et al. Amyloid-beta dynamics are regulated by orexin and the sleep-wake cycle. Science 2009 Nov;326(5955):1005–7.
[49] Liguori C, Romigi A, Nuccetelli M, Zannino S, Sancesario G, Martorana A, et al. Orexinergic system dysregulation, sleep impairment, and cognitive decline in Alzheimer disease. JAMA Neurol 2014 Dec;71(12):1498–505.
[50] Fronczek R, Overeem S, Lee SYY, Hegeman IM, van Pelt J, van Duinen SG, et al. Hypocretin (orexin) loss in Parkinson's disease. Brain 2007;130(6):1577–85.
[51] Long-Biao C, Bo-Wei L, Xiao-Hang J, Lin Z, Juan S. Progressive changes of orexin system in a rat model of 6-hydroxydopamineinduced Parkinson's disease. Neurosci Bull 2010;26(5):381–7.