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ORIGINAL RESEARCH

REM Density, Leg Movements, and REM Sleep without Atonia: Differentiating Type 1 from Type 2 Narcolepsy Through Polysomnographic Analysis, a Preliminary Study

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Objective: This study aimed to identify distinct REM sleep characteristics that differentiate type 1 narcolepsy (NT1) from type 2 narcolepsy (NT2) using polysomnography (PSG), while acknowledging the need for future validation against other hypersomnia disorders.

Methods: A retrospective review included 31 patients with NT1, 21 patients with NT2, and 24 healthy participants. Each participant underwent overnight PSG and a subsequent multiple sleep latency test (MSLT) to assess REM sleep parameters including average REM density, neck myoclonus index, and leg movement index. Cerebrospinal fluid (CSF) samples were collected to measure orexin-A and catecholamine levels.

Results: 1. NT1 patients demonstrated significantly higher average REM density versus NT2 ($P<0.05$); 2. Elevated REM sleep characteristics in NT1: neck myoclonus index (0.82 vs 0.25 n/hr), leg movement index (18 vs 7 n/hr), and REM sleep without atonia (RSWA) incidence (71% vs 24%) (all $P<0.05$); 3. Biochemical correlations: REM density negatively correlated with orexin-A ($r=-0.42$) and positively with norepinephrine ($r=0.38$) (both $P<0.05$).

Conclusion: While REM density, leg movement index and RSWA show promise for NT1/NT2 differentiation, these findings require validation in cohorts including idiopathic hypersomnia and other central hypersomnolence disorders. The observed electrophysiological patterns may reflect orexin-mediated dysregulation of REM motor control, but their diagnostic specificity remains to be established.

Keywords: narcolepsy, orexin-A, REM sleep without atonia, REM density, neck myoclonus

Introduction

Accurate subtyping of narcolepsy, a chronic sleep disorder, presents significant clinical challenges. While the International Classification of Sleep Disorders 3 (ICSD-3) categorizes narcolepsy into type 1 (NT1) with cataplexy and type 2 (NT2),[1] their differentiation remains problematic in clinical practice. NT2 exhibits most of the same symptoms as NT1, except for cataplexy. Studies show half of initially diagnosed NT2 patients are subsequently reclassified as NT1 or other sleep disorders, reflecting three key diagnostic limitations:[2] (1) Although cerebrospinal fluid (CSF) orexin testing shows high specificity, its invasiveness and cost restrict widespread use; (2) The multiple sleep latency test (MSLT) exhibits considerable intraday variability; (3) Some NT1 patients may lack typical cataplexy initially. This diagnostic uncertainty directly impacts treatment decisions, underscoring the urgent need for objective biomarkers.

Polysomnography (PSG) is a critical diagnostic tool used to identify sleep onset rapid eye movement periods (SOREM), a hallmark of narcolepsy. REM sleep abnormalities offer new insights for subtyping, but existing studies

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show inconsistencies. REM sleep without atonia (RSWA) is a prominent feature and a neurophysiological hallmark of abnormal rapid eye movement sleep behavior (RBD), reflecting nocturnal movement disorders in REM sleep.[3] Narcolepsy is the second most common cause of secondary RBD after α-synucleinopathies.[4] While NT1 patients demonstrate marked RSWA, this phenomenon also occurs in some NT2 cases,[4] implying that other intrinsic disease-related factors may be involved in the destabilization of REM sleep.

REM density is defined as the number of eye movements per minute during REM sleep.[5] Alterations in REM density have been suggested as a new biomarker for several brain disorders, with studies showing increased REM density in patients with depression[6] and decreased density in patients with Parkinson's.[7,8] REM density has also been studied in narcolepsy.[9] A large number of hypothalamic orexin neurons are lost in narcolepsy, especially in NT1.[10] Crucially, no study has systematically examined dose-response relationships between orexin levels and REM density changes—a knowledge gap hindering our understanding of REM regulation.

The role of catecholamine systems (dopamine, norepinephrine, etc) in narcolepsy remains debated. Researchers have drawn different or even opposite conclusions when measuring the dopamine level in the cerebrospinal fluid of narcolepsy patients,[11] recent large-scale research found no correlation with daytime sleepiness or REM abnormalities.[12] This paradox highlights two key questions: (1) Do distinct neurotransmitter patterns exist across subtypes? (2) Can REM microstructure reflect underlying neurochemical imbalances?

Addressing these gaps, our study integrates multimodal data with the following aims: (1) Identify PSG-based REM sleep characteristics (RSWA, REM density, leg movements) for distinguishing NT1 from NT2; (2) Quantify orexin-REM parameter relationships; (3) Characterize catecholamine profiles across subtypes. We hypothesize that patients with NT1 exhibit distinctive electrophysiological markers during REM sleep compared to NT2, including higher REM density, neck myoclonus index, leg movement index, and incidence of RSWA. These advances will significantly alleviate current diagnostic dilemmas that rely on invasive tests and subjective symptoms, laying the foundation for precision sleep medicine.

Materials and Methods

Subjects

We conducted a retrospective review of 31 patients with NT1 and 21 patients with NT2 who were admitted to the Affiliated Hospital of Shandong First Medical University between May 2021 and December 2023. Inclusion criteria were as follows: (1) adherence to healthy lifestyle habits:regular physical activity (≥150 minutes moderate or ≥75 minutes vigorous activity weekly); consumption of a balanced diet rich in fruits, vegetables, whole grains and lean proteins; avoidance of tobacco products, and limited alcohol consumption (≤1 drink/day for women, ≤2 for men). (2) absence of sedative, tonic, antiepileptic, or other sleep- or movement-affecting drugs in the preceding two months (Central nervous system stimulants including but not limited to amphetamines, methylphenidate, modafinil, sleep-affecting drugs, including benzodiazepines, non-benzodiazepine sedative-hypnotics, melatonin receptor agonists, orexin receptor antagonists, and prescription sedating antihistamines when used specifically for sleep regulation). (3) willingness to understand and cooperate with the trial, as evidenced by signing the informed consent form. Exclusion criteria included: Participants with the following medical conditions that can significantly affect sleep or daytime alertness were excluded: Cardiovascular diseases: including uncontrolled hypertension (BP > 140/90 mmHg despite treatment), heart failure (NYHA class II–IV), or severe arrhythmias. Neurological disorders: including epilepsy, Parkinson's disease, multiple sclerosis, or severe migraine (≥15 headache days/month). Metabolic/endocrine disorders: including uncontrolled diabetes (HbA1c > 8%), severe obesity (BMI ≥ 35 with comorbidities), or untreated hypothyroidism. Hematological disorders: including moderate-to-severe anemia (Hb < 10 g/dL for men, <9 g/dL for women) or chronic bleeding disorders. Other conditions: chronic kidney disease (eGFR < 60 mL/min/1.73m²), active cancer (except localized non-melanoma skin cancer), or chronic pain syndromes (eg, fibromyalgia), as well as apnea-hypopnea (AHI >5), circadian rhythm disturbances such as staying up late, engaging in shift work, or traveling through multiple time zones within the past week. 24 age and sex matched healthy controls without any sleep disorders or medication that could impact sleep in the

preceding two months were recruited. Before PSG+MSLT, all participants completed 7-day wrist actigraphy. The study received approval from the hospital ethics committee, and all participants provided written informed consent.

PSG

All participants underwent PSG (model: Grael) at the Clinical Sleep Medicine. The protocol included: Recording Protocol: Overnight monitoring (mean recording duration: 7.9 ±0.3 hours); Subsequent daytime MSLT (5-nap protocol); Synchronized infrared video recording. Physiological Monitoring: Neurological signals: 6-lead EEG (F3/M1, C3/M1, O1/M1, F4/M2, C4/M2, O2/M2) with impedance <5 kΩ; Bilateral EOG (outer canthus ±1 cm) sampled at 256 Hz; Submental EMG (2 cm above mandibular edge); ECG (lead II configuration); Respiratory monitoring: Nasal pressure transducers; Oro-nasal thermistors; Thoraco-abdominal belts; Pulse oximetry. Motor monitoring: Bilateral tibialis anterior EMG (3 cm inter-electrode distance); Neck myoclonus detection via supplementary sternocleidomastoid EMG. Scoring Methodology: All recordings were scored by two certified technicians (inter-rater κ=0.82) according to American Academy of Sleep Medicine (AASM) manual version 2.1. The PSG parameters included in this study were: sleep efficiency (SE), wakefulness after sleep onset (WASO), REM sleep latency (sleep onset to first REM), REM sleep duration, REM sleep percentage, average REM density, neck myoclonus index, leg movement index and the presence or absence of RSWA. Quality Control: Signal quality verified hourly (noise <10μV for EEG/EOG); Video-PSG synchronization verified (±100ms tolerance); Movement artifacts automatically flagged and manually reviewed.

Diagnostic Criteria for Narcolepsy Type 1 is: Both of the following criteria must be met: 1. Daily occurrences of irresistible drowsiness or unintended daytime sleep episodes, with symptoms persisting for at least 3 months. 2. Fulfillment of one or both of the following conditions: (1) Presence of cataplexy (meeting the basic characteristics of the definition), and a mean sleep latency of ≤8 minutes on a standard Multiple Sleep Latency Test (MSLT), with the occurrence of ≥2 sleep-onset REM periods (SOREMPs). If a nocturnal polysomnography (nPSG) is conducted prior to the MSLT, the presence of a SOREMP (REM period occurring within 15 minutes of sleep onset) can substitute for one SOREMP in the daytime MSLT. (2) Detection of cerebrospinal fluid (CSF) hypocretin-1 (Hcrt-1) levels ≤110 pg/mL or less than one-third of the average value of normal subjects tested under the same standards. Diagnostic Criteria for Narcolepsy Type 2 is: all five of the following criteria must be met: 1. Daily occurrences of irresistible drowsiness or unintended daytime sleep episodes, with symptoms persisting for at least 3 months. 2. A mean sleep latency of <8 minutes on a standard Multiple Sleep Latency Test (MSLT), with the occurrence of ≥2 sleep-onset REM periods (SOREMPs). If nocturnal polysomnography (nPSG) is conducted prior to the MSLT (ensuring at least 6 hours of sleep), the presence of a SOREMP (REM period occurring within 15 minutes of sleep onset) can substitute for one SOREMP in the daytime MSLT. 3. Absence of cataplexy. 4. CSF hypocretin-1 (Hcrt-1) levels, as measured by radioimmunoassay, are >110 pg/mL or greater than one-third of the average value of normal subjects tested under the same standards. 5. The symptoms of sleepiness and/or MSLT results cannot be explained by other causes, such as insufficient sleep, obstructive sleep apnea (OSA), delayed sleep phase disorder, medication use, or withdrawal.[1]

REM density was calculated as the number of REM cycles/REM period time (min), with a single REM defined as an EOG lead showing a conjugate, irregular, sharply peaked ocular motility wave recorded in the EOG leads, with an initial deflection time to peak usually <500 ms. Neck myoclonus is characterized by rapid twitching or twisting of the neck during sleep. Neck myoclonus index was calculated as the number of Neck myoclonus/REM period time (hr). RSWA was calculated as: Tonic activity: Percentage of 3-second mini-epochs in REM sleep with EMG amplitude exceeding 2× the background EMG amplitude of NREM sleep. Phasic activity: Percentage of 30-second REM sleep epochs containing bursts of EMG activity (0.1–5.0 s duration, >4× background amplitude).

Scales Used

The scales used in this study include the Pittsburgh Sleep Quality Index (PSQI),[13] the Epworth Sleepiness Scale (ESS),[14] the Hamilton Anxiety Scale (HAMA), and the Hamilton Depression Scale (HAMD).[15] Copyright permissions for ESS (License No. 118373), HAMA, and HAMD were obtained from MAPI, and for PSQI from the University of Pittsburgh Sleep and Circadian Science Center. All scales were administered by the same trained attending neurologist and reviewed by a psychologist.

CSF Orexin-A and Catecholamine Measurement

NT1and NT2 patients underwent lumbar puncture. All patients provided informed consent (patients under 18 years of age had consent provided by their legal guardian or legally authorized representative). Due to the invasive nature of the procedure, the control group declined lumbar puncture. CSF samples were promptly stored at 4 °C and directly sent from the sleep center to Jinan KingMed Diagnostics for analysis. CSF orexin-A levels were quantified using a radioimmunoassay (RIA) kit from Phoenix Pharma. Catecholamine levels were determined utilizing high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS).

Sample Size Calculation

The sample size was calculated using PASS software (v21.0) with the "One-Way Analysis of Variance F-Tests" module, based on preliminary data for our primary outcome measure, REM density. Assuming group means of 5.27 (NT1), 2.65 (NT2), and 7.20 (controls) with a common standard deviation of 3.69, an α level of 0.05, and a target power of 80%, the analysis indicated that 21 participants per group would achieve 94.8% power to detect significant between-group differences. Our final enrollment exceeded this threshold (NT1=31, NT2=21, controls=24), ensuring adequate statistical power for the primary comparisons.

Statistical Analyses

Data were normalized using IBM SPSS Statistics 25. Continuous variables were assessed for normality using the Shapiro–Wilk (S-W) test. If normally distributed, differences between the three groups were analyzed using one-way repeated measures ANOVA. Post hoc pairwise comparisons were conducted, and the results were adjusted using Bonferroni correction (for three-group comparisons). Mean values with standard deviation were reported. For non-normally distributed data, Friedman test was utilized. Post hoc pairwise comparisons were performed, and Bonferroni correction was applied. ROC-derived thresholds were cross-validated via bootstrapping to assess stability. The Youden index was used to select optimal cut-offs. Results were reported as median with interquartile range. Pearson's correlation was used for correlation analysis. Statistical significance was set at $P<0.05$ (two-sided).

Result

Demographic Data and Scales

Seventy-six individuals were ultimately included in the study, with 31 NT1 patients (23 males), 21 NT2 patients (13 males), and 24 healthy controls (13 males). There were no statistical differences in gender, age, BMI, HAMA and HAMD scores among the three groups. PSQI and ESS scores were higher in the NT group compared to the control group (Table 1).

Table 1 Comparison of Clinical Data and Scales Among the Three Groups

	A=NT1 (n= 31)	B=NT2 (n= 21)	C=Control (n= 24)	Statistic	*P*	Post-Hoc
Age (years)	22 (17, 37)	17 (15, 21)	25 (24, 26)	5.04	0.081	
Sex (M/F)	23/8	13 /8	13/11	2.45	0.293	
BMI	27.3 ± 4.6	24.7 ± 5.0	25.2 ± 4.4	0.85	0.433	
PSQI (score)	6.0 (4.0, 8.8)	5.0 (4.0, 6.0)	3.0 (2.3, 4.0)	12.21	0.002*	A>C
ESS (score)	16 (14, 18)	15 (12, 17)	4 (1, 5)	31.13	<0.001*	A, B>C
HAMA (score)	10 (6, 15)	17 (10, 25)	9 (6, 14)	4.01	0.134	
HAMD (score)	5.0 (4.0, 8.0)	8.5 (6.3, 10.0)	6 (5, 8)	23.14	0.208	

Notes: *P<0.05. A: NT1, B: NT2, C: control. Continuous data presented as mean ± standard deviation unless otherwise noted as median (interquartile range, IQR).

Abbreviations: PSQI, Pittsburgh Sleep Quality Index; ESS, Epworth Sleepiness Scale; HAMA, Hamilton Anxiety Scale; HAMD, Hamilton Depression Scale.

PSG Data

No statistically significant differences in REM sleep latency were found among the three groups. The NT1 group showed lower sleep efficiency compared to the control group and NT2 group, NT1 patients experienced prolonged wakefulness after falling asleep, indicating poor sleep at night. The REM sleep time and proportion for the NT1 and NT2 group were higher than those of the control group. Notably, REM density was higher in NT1 than in NT2 (cut-off: 2.44/min, AUC = 0.806, 95% CI: 0.672–0.902, sensitivity = 61.90%, specificity = 90.32%, Youden index = 0.522), suggesting its potential as a discriminative biomarker for NT1 (Table 2).

Neck myoclonus index and leg movement index in REM sleep were significantly elevated in NT1 compared to NT2 and controls ($p < 0.05$). Leg movement index in REM sleep demonstrated moderate discriminative power (cut-off: 15.80/hr, AUC = 0.683, 95% CI: 0.539–0.805, sensitivity = 90.48%, specificity = 54.84%). Neck myoclonus index showed weaker but suggestive performance (cut-off: 0.51/hr, AUC = 0.619, 95% CI: 0.474–0.750) (Table 2). RSWA and total leg movement index were also higher in NT1, further supporting their utility in distinguishing NT1 from NT2 (Table 3).

Due to the refusal of lumbar puncture in the control group, this study only compared the levels of orexin-A, dopamine (DA), NE, and E in the CSF of NT1 and NT2 group. The results showed that the level of orexin-A of the NT1 group was significantly reduced, and the level of E was also lower than NT2. There were no statistical differences in the levels of DA and NE (Table 4).

Association Between REM Sleep Characteristics and Clinical Symptoms

In order to gain a better understanding of the relationship between REM sleep characteristics and clinical symptoms in NT patients, we conducted a comparison of the average REM density, neck myoclonus index, and leg movement index with cataplexy, hallucinations, sleep paralysis, RBD, and nightmares.

The results revealed that the leg movement index in REM sleep was elevated in NT patients with RBD, average REM density was elevated in NT patients with cataplexy and hallucinations (Figure 1).

Table 2 Discriminative Performance of REM Sleep Characteristics for NT1 Diagnosis

	AUC	*P*	95% CI	Cut-Off	Sensitivity (%)	Specificity (%)	Youden Index
Average REM density (n/min)	0.806	<0.001*	0.672–0.902	2.44	61.90	90.32	0.522
Leg movement index in REM sleep (n/hr)	0.683	0.014*	0.539–0.805	15.80	90.48	54.84	0.453
Neck myoclonus index (n/hr)	0.619	0.150	0.474–0.750	0.51	76.19	61.29	0.375

Notes: *P<0.05. AUC values and cut-offs were derived from ROC analysis with bootstrapping validation.

Table 3 Comparison of PSG Data Among the Three Groups

	A=NT1 (n= 31)	B=NT2 (n= 21)	C=Control (n= 24)	Statistic	*P*	Post-Hoc
Sleep efficiency (%)	86 (73, 90)	91 (85, 96)	92 (83, 96)	7.08	0.029*	A<B, C
REM sleep latency (min)	73.8±100.4	115.3±103.4	121.5± 59.1	2.187	0.120	
REM sleep time (min)	88.8±30.8	104.3±55.6	65.3±24.8	5.933	0.004*	A, B>C
REM sleep proportion (%)	20.3±5.6	20.6 ±7.3	14.7 ±5.2	7.273	0.001*	A, B>C
WASO (min)	86.76±57.59	61.76±75.32	41.13±34.62	4.334	0.017*	A>C
Average REM density (n/min)	4.3 (3.3, 6.3)	2.3 (1.7, 4.3)	7.3 (2.7, 11.0)	8.58	0.014*	C>A>B
Neck myoclonus index (n/hr)	0.82 (0.24, 1.33)	0.25 (0.12, 0.51)	0.12 (0.00, 0.25)	8.58	0.014*	A>C
Leg movement index in REM sleep (n/hr)	18 (7, 36)	7 (4, 13)	3 (1, 8)	19.49	<0.001*	A>B, C
Total leg movement index (n/hr)	18 (9, 31)	8 (6, 15)	5 (2, 11)	19.09	<0.001*	A>B, C
RSWA (±)	22/9	5/16	4/20	13.017	0.000*	A>B, C

Notes: *P<0.05. A: NT1, B: NT2, C: control. Continuous data presented as mean ± standard deviation unless otherwise noted as median (interquartile range, IQR).
Abbreviations: WSAO, wakefulness after sleep onset; RSWA, REM sleep without atonia.

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Table 4 Comparison of Levels of Orexin-A and Catecholamines in CSF Between NT1 and NT2 Group

	A=NT1 (n= 31)	B=NT2 (n= 21)	Statistic	P
Orexin-A (pmol/L)	19 (14, 34)	209 (173, 236)	36.88	<0.001*
DA (pmol/L)	27 (21, 35)	20 (16, 33)	0.82	0.365
NE (pmol/L)	392 (300, 589)	323 (256, 388)	2.74	0.098
E (pmol/L)	0.95 (0.51, 1.97)	2.10 (1.61, 2.69)	4.77	0.029*

Notes: *P<0.05. Non-normally distributed data are presented as median (interquartile range, IQR).
Abbreviations: DA, dopamine; NE, norepinephrine; E, epinephrine.

Correlation Between REM Sleep Characteristics and Neurochemical Markers

Finally, we investigated the correlation between REM sleep characteristics and orexin-A, catecholamines. Our findings revealed that, with the exception of the neck myoclonus index, other characteristics of the REM sleep, such as REM

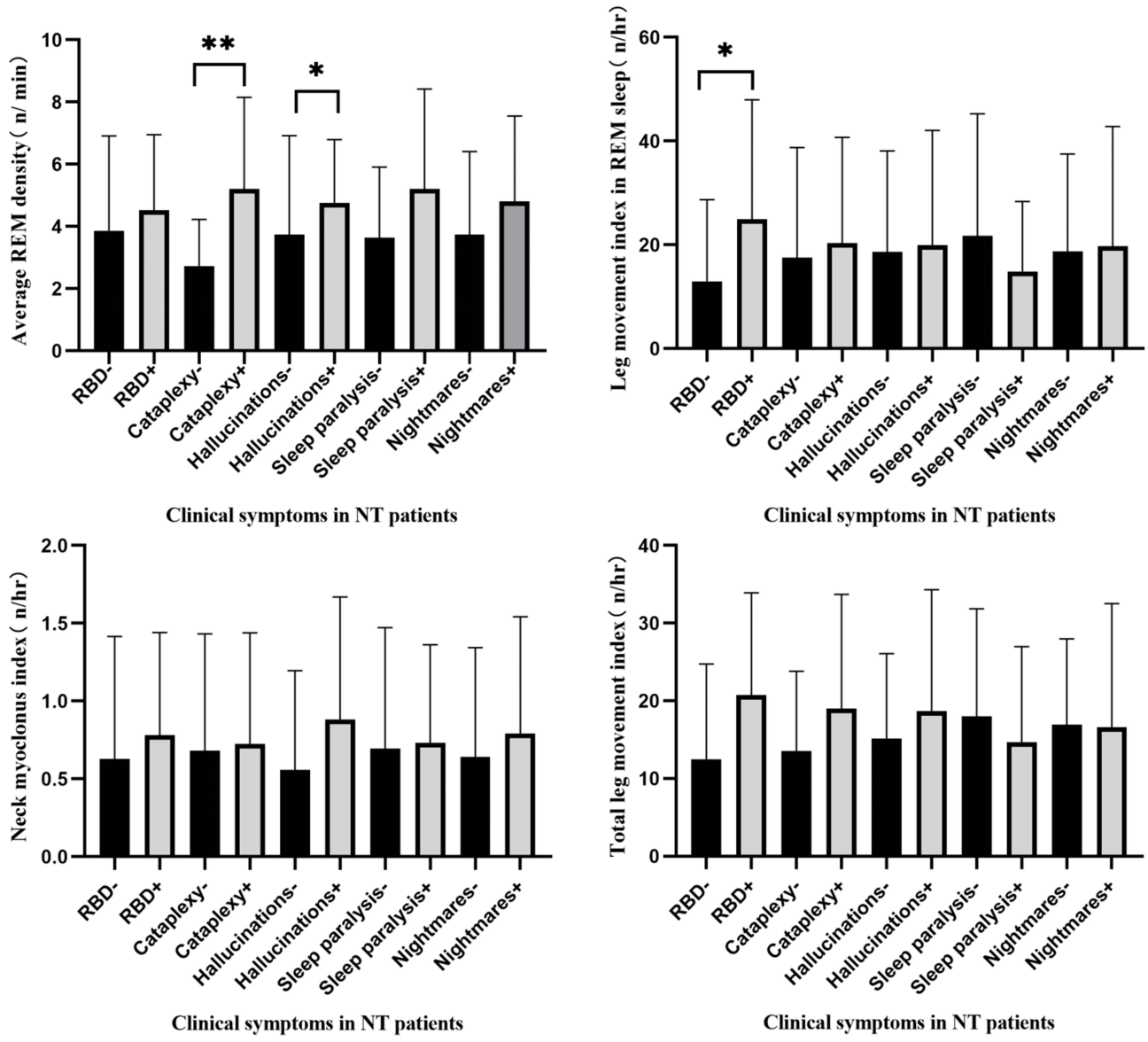

Figure 1 Relationship between REM sleep characteristics and clinical symptoms in NT patients (*P<0.05, **P<0.01).

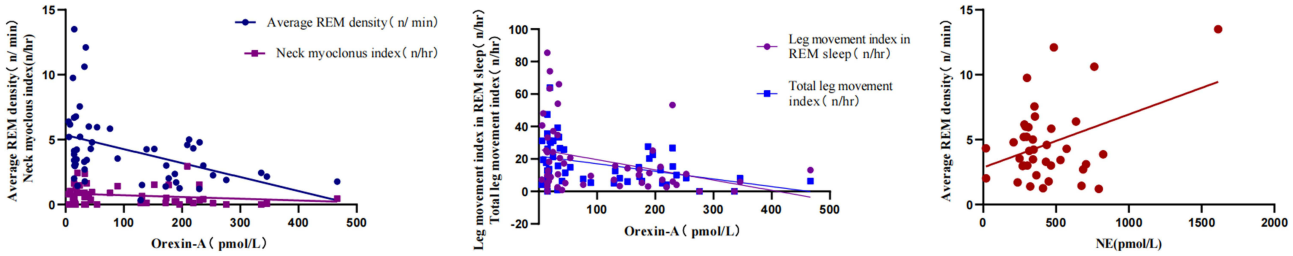

Figure 2 Correlation of REM sleep characteristics with orexin-A and catecholamines in NT patients.

density and leg movements, exhibited a negative correlation with orexin-A levels. This suggests that lower levels of orexin-A were associated with increased eyes movements and leg movements during the REM sleep. Additionally, there was a positive correlation between the average REM density and norepinephrine (NE) levels, indicating that higher NE levels were associated with more eye movements during the REM sleep (Figure 2).

Discussion

In this study, we analyzed the characteristics of REM sleep and the levels of orexin and catecholamines in patients with narcolepsy, and we studied the relationship between REM sleep characteristics and the clinical symptoms.

REM Sleep Characteristics

The results of our study showed that the NT1 group had a longer time of wakefulness after sleep onset and lower sleep efficiency, suggesting that NT1 patients had poor nighttime sleep. This is consistent with the results of previous studies.[16] Our study focused on REM sleep characteristics.

Average REM density: We found that the average REM density was significantly higher in the NT1 group than in the NT2 group. At a cut-off value of 2.44/min, REM density demonstrated strong discriminative power for NT1 diagnosis, suggesting its utility as an electrophysiological marker to distinguish NT1 from NT2. The direction of eye movements during REM has been shown to be consistent with head direction (HD) neurons in the thalamus,[17] known as the "scanning hypothesis" of rapid eye movements during REM sleep.[18] However, this theory has not been unanimously accepted. A review states that REM density may be a measure of sleep need, reflecting the amount of prior accumulated sleep and that it may serve as an index of "sleep satiety".[6] Vanková compared REM density in patients with NT1 and idiopathic hypersomnia, and also found that REM density was elevated in patients with NT1,[9] which is in agreement with our results, but they did not set up a healthy control group. Another study showed that REM density was higher in patients with narcolepsy than in patients with RBD and healthy controls.[19] Recent research suggests that, elevated REM density during REM sleep may be a key correlate of RSWA in NT patients.[20]

While our findings on REM density differences between NT1 and NT2 are statistically significant, several conceptual boundaries must be emphasized: (1) The diagnostic specificity of these markers remains undefined against idiopathic hypersomnia or sleep deprivation—conditions that frequently mimic NT2 in clinical practice. (2) The observed higher REM density in controls versus NT1/NT2 likely reflects methodological and physiological factors. Controls' shorter REM duration (65.3 vs 88.8–104.3 min in patients) may artifactually inflate density calculations, while EMG artifacts in patients could obscure EOG signals. The control group in this study may include students who involve high-load spatial attention tasks. Existing evidence suggests that such cognitive activities can specifically increase REM duration and density.[21] Despite this, REM density effectively discriminates NT1 from NT2, warranting further validation in comorbid populations. This paradox underscores the need for standardized measurement protocols when applying REM density as a diagnostic parameter.

RWSA and leg movement index: The NT1 group had higher RSWA rate compared to NT2 group. This finding is consistent with previous studies.[22–24] Recent animal studies indicate that a specific subset of orexin-producing neurons in the hypothalamus plays a crucial role in regulating REM sleep patterns through their projections to the sublaterodorsal tegmental nucleus in the brainstem.[25] Experimental suppression of these particular REM-active orexin neurons in rodent

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models has been shown to disrupt normal REM sleep organization, potentially offering a mechanistic explanation for the REM sleep abnormalities observed in narcolepsy patients.[26]

Neck myoclonus index: We observed that the neck myoclonus index was elevated in NT patients compared to controls, aligning with our earlier findings.[24] Nevertheless, there are limited studies on the mechanism of neck myoclonus. Recent studies have shown that changes in heart rate variability precede the onset of neck myoclonus, suggesting that autonomic function may play a role in its regulation.[27] However, when examining the correlation of the neck myoclonus index with the orexin system and norepinephrine, no statistically significant differences were found. Further investigation is needed to understand the specific mechanism of neck myoclonus function.

Orexin-A and Catecholamines in NT Patients

Our findings revealed no significant differences in dopamine (DA) and norepinephrine (NE) levels in NT1and NT2 patients, while NT1 patients had lower orexin-A and epinephrine (E) levels. Dopaminergic system, which projects to orexin neurons, is the neurobiological basis of narcolepsy in animals and humans. In our study, as the control group did not undergo lumbar puncture, we were unable to compare the differences in DA levels between NT patients and normal individuals. However, there was no statistically significant difference in DA levels between NT1 and NT2 patients. A study compared the serum metabolites between NT1 patients and controls, they found epinephrine displayed a significant downward trend.[28] In NT and idiopathic hypersomnolence patients, twenty-four hour urinary epinephrine (E) plus norepinephrine (NE) was higher than normal.[29] As our research has shown, the level of epinephrine (E) in CSF of NT1 patients is lower than NT2 patients, suggesting that epinephrine (E) may be a potential biomarker for the diagnosis of NT1, especially in urine, as lumbar puncture is an invasive examination.

REM Sleep Characteristics and Clinical Symptoms in NT Patients

Our results revealed that the leg movement index in REM sleep was elevated in NT patients with RBD, average REM density was elevated in NT patients with cataplexy and hallucinations. Furthermore, REM density and leg movements, exhibited a negative correlation with orexin-A levels and there was a positive correlation between the average REM density and norepinephrine (NE) levels.

As mentioned earlier, reduction in orexin secretion inhibited the effect of the brainstem inhibitory zone on REM muscle tone leading to a failure in REM atonia.[25] REM sleep without atonia not only manifest in jaw muscle, but also in leg muscles, leading to an increase in leg movements. Some studies have confirmed that orexin deficiency causes a functional defect in the motor control involved in the development of cataplexy during wakefulness and RBD/RSWA/phasic motor activity during REM sleep.[30,31] This is consistent with our research.

Average REM density in NT patients with cataplexy was elevated, lower levels of orexin-A were associated with increased eyes movements. This suggests that there may be a common mechanism between cataplexy and REM density. The neurochemistry of cataplexy, originally focused on catecholamines and acetylcholine now extend to orexin and other neuromodulator.[32,33] Coincidentally, eyes movements are directly or indirectly controlled by the orexin system.[34] So, the decrease in orexin may be the common mechanism for both cataplexy and eyes movements. Hallucinations occurred more frequently and with more motor and multimodal aspects in narcolepsy with cataplexy than in narcolepsy without cataplexy, the risk factors for hallucinations were sleep paralysis and RBD.[35] There is currently no research on hallucinations and REM density, but our study shows that REM density is elevated in NT patients with hallucinations. The mechanism behind this remains to be further studied.

We found there was a positive correlation between the average REM density and NE levels. While studies have shown that NE levels are decreased in patients with PD,[36] as is REM density,[8] no direct relationship between NE and REM density has been established. The role of adrenergic signaling in REM sleep modulation appears multifaceted. Current theories suggest two potential mechanisms:[8] first, the progressive decline in NE/E levels during NREM sleep may reduce adrenergic tone, thereby facilitating REM onset. Alternatively, REM sleep may require NE/E concentrations to remain within a specific narrow range- neither too high nor completely absent- as both extremes could suppress REM phenomena. However, the specific pathways through which noradrenergic systems control ocular activity in REM sleep have yet to be fully elucidated.

The correlations between REM density/leg movements and clinical symptoms (cataplexy, hallucinations) should be interpreted with caution. While our strict exclusion criteria enhanced internal validity by minimizing confounding factors, they significantly limit the clinical generalizability of our findings. According to our clinic's electronic health records, typical narcolepsy patients often present with comorbidities: psychiatric conditions (primarily depression), sleep-disordered breathing (AHI≥5), and chronic pain or migraines. This contrasts sharply with our study sample, suggesting our results may not fully reflect real-world clinical populations. Important unaddressed interactions include: the impact of antidepressants on REM metrics (with literature suggesting SSRIs may reduce REM density),[37] potential OSA-related modifications of REM microstructure, and the influence of pain-related arousals on sleep continuity. Consequently, we recommend clinicians exercise caution when applying these findings: (1) for patients with comorbidities, gold-standard tests like CSF orexin measurement should remain primary; (2) if utilizing REM density measures, clinicians should account for SSRI effects and consider that untreated OSA may exaggerate RSWA; and (3) future research must prioritize real-world cohort studies that include patients with these common comorbidities to properly evaluate the robustness of these potential biomarkers.

Limitations

While this study provides novel insights into electrophysiological differences between NT1 and NT2, several important limitations must be acknowledged. The most critical limitation is the absence of comparator groups with idiopathic hypersomnia and other central disorders of hypersomnolence, which fundamentally restricts our understanding of the biomarkers' specificity and clinical utility in real-world differential diagnosis. Our findings are further constrained by the modest sample size (While the study achieved high statistical power for primary REM density comparisons, power for secondary analyses may be limited due to smaller subgroup sizes) and single-center recruitment, potentially limiting generalizability to atypical phenotypes and ethnically diverse populations. The retrospective design prevented standardization of treatment protocols and longitudinal monitoring, while strict exclusion of common comorbidities (eg, depression, OSA) may reduce applicability to complex clinical cases. Technical challenges included inter-scorer variability in PSG interpretation and high refusal rates for CSF collection in controls, raising questions about clinical feasibility. Most importantly, the observed association between norepinephrine and REM density may reflect compensatory adaptation rather than direct causation, and the pathophysiological basis of neck myoclonus remains unclear. These limitations collectively suggest that while our findings advance the understanding of narcolepsy subtypes, they should be considered hypothesis-generating rather than clinically actionable. Future multicenter studies with larger, more diverse cohorts that include other hypersomnia disorders and comorbid patients are essential to validate these biomarkers and establish their diagnostic utility.

Conclusion

These findings establish preliminary electrophysiological distinctions between NT1 and NT2, though definitive validation requires: Larger prospective studies, Inclusion of comparator hypersomnia groups (idiopathic hypersomnia, sleep-deprived controls), Real-world testing in comorbid populations. Until such validation is achieved, we propose a cautious, tiered approach: Tier 1: Use REM density/RSWA as adjunctive markers for discordant MSLT-clinical cases. Tier 2: For NT2 patients with elevated REM density: Serial PSG monitoring (6–12 month intervals); CSF orexin testing if progression risk factors emerge. These biomarkers should not be used in isolation for clinical decision-making. The current evidence primarily advances pathophysiological understanding rather than immediate diagnostic utility.

Data Sharing Statement

The data that support the findings of this study are available from the corresponding author, Weiwei Huang, upon reasonable request.

Ethical Approval

This study was approved by the Human Research Ethics committee of the First Affiliated Hospital of Shandong First Medical University & Shandong Provincial Qianfoshan Hospital. (Ethics approval number: YXLL-KY-2021-026). The guidelines outlined in the Declaration of Helsinki were followed.

Author Contributions

Juanjuan Xu: Writing – review & editing, Writing original draft, Validation, Formal analysis; Data curation. Shanshan Lu: Writing – review & editing, Data curation, Conceptualization; Yanxia Zhang, Kejun Zang: Writing – review & editing, Resources, Data curation; Wanyu Zhao: Writing – review & editing, Project administration, Methodology, Investigation; Zian Yan, Jiyou Tang: Writing – review & editing, Software, Investigation. Weiwei Huang: Writing – review & editing, Supervision, Resources, Project administration, Methodology, Conceptualization. All authors gave final approval of the version to be published and have agreed on the journal to which the article has been submitted; and agreed to be accountable for all aspects of the work.

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