공학석사 학위논문

Changes in Physiological Network Connectivity of Body System in Narcolepsy during REM Sleep

렘 수면 중 기면 환자의 인체 시스템 내 생리학적 네트워크 연결성 변화

2022년 02월

서울대학교 대학원
협동과정 바이오엔지니어링 전공
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Master Dissertation

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Interdisciplinary Program in Bioengineering
The Graduate School
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Abstract

Changes in Physiological Network Connectivity of Body System in Narcolepsy during REM Sleep

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Background: Narcolepsy is marked by pathologic symptoms including excessive daytime drowsiness and lethargy, even with sufficient nocturnal sleep. There are two types of narcolepsy: type 1 (with cataplexy) and type 2 (without cataplexy). Unlike type 1, for which hypocretin is a biomarker, type 2 narcolepsy has no adequate biomarker to identify the causality of narcoleptic phenomenon. Therefore, we aimed to establish new biomarkers for narcolepsy using the body's systemic networks.

Method: Thirty participants (15 with type 2 narcolepsy, 15 healthy controls) were included. We used the time delay stability (TDS) method to examine temporal information and determine relationships among multiple signals. We quantified and analyzed the network connectivity of nine biosignals (brainwaves, cardiac and respiratory information, muscle and eye movements) during nocturnal sleep. In particular, we focused on the differences in network connectivity between groups according to sleep stages and investigated whether the differences could be potential biomarkers to classify both groups by using a support vector machine.

Result: In rapid eye movement sleep, the narcolepsy group displayed more connections than the control group (narcolepsy connections: 24.47 ± 2.87, control connections: 21.34 ± 3.49; $p = 0.022$). The differences were observed in movement and cardiac activity. The performance of the classifier based on connectivity differences was a 0.93 for sensitivity, specificity and accuracy, respectively.

Conclusion: Network connectivity with the TDS method may be used as a biomarker to identify differences in the systemic networks of patients with narcolepsy type 2 and healthy controls.

Keyword: Sleep, Narcolepsy, Narcolepsy type 2, Brain, Connectivity, REM, Time delay stability

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List of Abbreviations

AASM	American academy of sleep medicine
AHI	Apnea hypopnea index
AI	Apnea index
ANOVA	Analysis of variance
CSF	Cerebrospinal fluid
DS	Deep sleep
ECG	Electrocardiogram
EEG	Electroencephalogram
EMG	Electromyogram
EOG	Electrooculogram
FFT	Fast fourier transform
HF	High-frequency
HI	Hypopnea index
HR	Heart rate
HRV	Heart rate variability
IRB	Institutional review board
LF	Low-frequency
LF/HF	Ratio of LF and HF
LS	Light sleep
MSLT	Multiple sleep latency test
N.S.	Not significant

OPA	Ocular pulse amplitude
OSA	Obstructive sleep apnea
PLMD	Periodic limb movement disorder
PLMI	Periodic limb movement index
pNN50	Percentage of successive RRI differing by more than 50 ms
PSG	Polysomnography
REM	Rapid eye movement
RMSSD	Root mean square of successive RR interval differences
RRI	R-peaks of consecutive beats
RSWA	REM sleep without atonia
SDNN	Standard deviation of RR intervals
SE	Sleep efficiency
SOL	Sleep onset latency
SOREMP	Sleep onset REM period
SVM	Support vector machine
SWS	Slow wave sleep
TDS	Time delay stability
TRT	Total recording time
TST	Total sleep time
TWT	Total wake time
WASO	Wake after sleep onset

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Introduction

1.1. Narcolepsy

Narcolepsy is a neurological disease characterized by excessive daytime drowsiness and lethargy, hallucination, lowered concentration, and dysfunction of the brain's ability to control the sleep-wake cycle **[1]**. These symptoms can not only affect individuals' ability to work but also lower their quality of life **[2]**. Narcolepsy is known to be related to rapid eye movement (REM) sleep and the brain's control of this state. Patients with narcolepsy fall into REM sleep suddenly in the daytime and fall into REM sleep much faster at night, with REM sleep accounting for a higher proportion of total sleep compared to healthy controls **[3-6]**. These clinical differences imply that REM sleep is a crucial indicator for distinguishing narcolepsy.

Narcolepsy is divided into two types: type 1 (with cataplexy) and type 2 (without cataplexy). Both types present with symptoms of excessive daytime drowsiness, but type 1 involves cataplexy, which causes sudden REM sleep in the daytime. In addition, type 1 has a specific biomarker: hypocretin in the cerebrospinal

fluid (CSF), which chemically controls the nervous and endocrine systems, is used to identify the cause of narcolepsy **[6]**. A hypocretin deficiency affects the body's ability to control the nervous and endocrine systems, leading to the symptoms of narcolepsy and cataplexy. On the other hand, narcolepsy type 2 does not have specific biomarkers to explain the causality of the pathological phenomenon **[4,7]**. Therefore, it is essential to identify biomarkers to interpret the symptoms of patients with narcolepsy type 2. To establish new biomarkers, we focused on the interactions of the body's neurological and electronic systems and examined the differences between patients with narcolepsy and healthy controls. For this, it was necessary to quantify physiological differences in the body's network between patients with narcolepsy and controls with understandable metrics. (Narcolepsy type 2 will be referred to as "narcolepsy" from now on.)

1.2. Physiological interactions in body system

We analyzed the differences between patients with narcolepsy and healthy controls by focusing on the interactions of the physiological system during nocturnal sleep. Physiological systems continuously interact within organs and coordinate their functions through a feedback mechanism with variance of time. The interactions change according to the regulatory effect of the nervous system, physiological states, and pathological conditions **[8-9]**. Numerous studies have sought to determine the physiological interactions of the human body during sleep. Studies on interactions between the brain and heart show that brain-heart and brain-brain interactions have their own causal directions and that brainwave and heart rate variability (HRV) components affect each other **[10]**. Studies on cardiopulmonary interaction, which represents the connection between the heart and respiration, have revealed different aspects according to varying pathologic conditions. Unidirectional coupling from breathing to cardiac activity increases during the wake state and during REM sleep in patients with obstructive sleep apnea (OSA) when compared to controls **[11]**. Moreover, relative to controls, patients with depression showed a reduction in high-frequency cardiopulmonary coupling and increment low-frequency coupling, both of which indicate an unstable sleep state **[12]**. In research on insomnia, patients showed a decreased linear relationship and coherence between delta brainwave power and high-frequency power of HRV compared to controls **[13]**. These studies indicate that clinical and pathological differences exist within interactions in the human body. However, no proper studies, especially on narcolepsy type 2, have examined physiological interactions between patients with narcolepsy and controls from the aspect of systemic networks. Several studies have shown a partial

interaction between the brain and peripheral organs and differences in the sleep structures and characteristics of REM sleep. It has been reported that the cortical circuits of patients with narcolepsy dissociate motor components of the body during REM sleep, leading to confusion between dreams and reality **[14]**. Research has shown that, compared to healthy individuals, patients with narcolepsy show bursts of theta rhythm brainwaves **[15]** and frequent movement of the submentalis muscle **[16]** during REM sleep. As both patients with narcolepsy and controls show clinical differences and experience pathologically different conditions and states during sleep, there may be differences in both groups regarding the physiological interactions of systemic networks. Therefore, we hypothesized that interactions in the physiological systems of patients with narcolepsy differ significantly in REM sleep, as do the sleep structures and characteristics.

1.3. Connectivity with time delay stability

Determining the differences in complex interactions within the brain and peripheral sites is fundamental to analyzing and interpreting interactions between narcolepsy and control groups **[17]**. We examined connectivity among biosignals within the body to quantify and compare the strength of the connections. By analyzing connectivity, relationships among biosignals that represent characteristics of different body parts can be viewed as a network. Connectivity among biosignals can be estimated via strength and stability, and the overall systemic connectivity can represent the physiological state. Considering the time-variant and dynamic characteristics of the system, we used the time delay stability (TDS) method based on cross-correlation to quantify the connectivity using time. Although there are numerous ways to analyze network connectivity, we chose the TDS method because it is sensitive to temporal resolution and can be used to address the complex relationships of multiple signals **[8]**. The TDS method can also be used to calculate correlations between signals, and it is useful for determining stability with temporal information. Thus, the TDS method can be used to compare connectivity among multiple biosignals according to sleep stages and may also be used to explain these physiologically complex systems.

Using the TDS method, we compared the connectivity of biosignal networks in patients with narcolepsy and healthy controls during nighttime sleep **[8,9,18]**. We selected data from polysomnography focused on the characteristics of narcoleptic symptoms, including leg, chin and eye movements, cardiopulmonary function, and electroencephalogram (EEG) information. With reference to sleep stages recorded using polysomnography (PSG), we applied the TDS method to selected biosignals

for each stage of nocturnal sleep and compared the two groups. We evaluated all possible connections to analyze the brain and peripheral feature connectivity for each sleep stage. We aimed to evaluate the differences in connectivity in each sleep stage and to determine the specific causality of physiological differences between patients with narcolepsy and healthy controls **[19]**.

1.4. Dissertation Outline

This thesis consists of following chapters.

- Chapter 2 presents experimental environment and participants with computational methods for analysis.
- Chapter 3 describes results of our study and delineates interpretations of the results as a discussion.
- Chapter 4 summarizes limitations from the overall process of our study and proposes suggestions.

This thesis is based on following scientific article that have been accepted for publication:

> D.Y. Son, et al. Changes in physiological network connectivity of body system in narcolepsy during REM sleep. *Computers in Biology and Medicine*. 2021;136. https://doi.org/10.1016/j.compbiomed.2021.104762

The author of this thesis contributed to the above study as follows: conception and design of the experiments; data acquisition, analysis, and interpretations; and wrote and reviewed the manuscript.

2

Material and Methods

2.1. Participants

Twenty patients with narcolepsy type 2 and 15 healthy controls were included. We excluded individuals with any sleep disorders other than cataplexy from the narcolepsy group. Five individuals in the narcolepsy group who had obstructive sleep apnea (OSA), periodic limb movement disorder (PLMD), depression, schizophrenia, epilepsy, or physical or mental problems were excluded. Those taking medications that could affect sleep were also excluded. Those diagnosed with moderate or severe OSA and PLMD were excluded (apnea hypopnea index (AHI) > 15 times, periodic limb movement index (PLMI) > 25 times) **[20-30]**. Control group participants also satisfied the above-mentioned inclusion criteria, except for the

narcolepsy criteria. After exclusion, 15 patients with narcolepsy (8 men and 7 women; aged 24.8 ± 8.23 years) and 15 controls (7 men and 8 women; aged 27.67 ± 3.84 years) were included in the analysis **[18,31,32,33]**. All participants underwent nighttime PSG, and the narcolepsy group underwent a daytime multiple sleep latency test (MSLT) the day after PSG. Patients with narcolepsy satisfied the American Academy of Sleep Medicine (AASM) criteria **[34]** for narcolepsy (average sleep onset latency for all MSLTs ≤ 8 min and number of sleep onset REM in all MSLTs ≥ 2) and were diagnosed **[35]** by a clinician from Seoul National University Hospital in South Korea (**Table 2-1**). This study was approved by the Institutional Review Board of Seoul National University Hospital (IRB No. 2101-120-1190).

Table 2-1. PSG information for participants with narcolepsy and controls

	Narcolepsy	**Controls**	**P**
In PSG	**(N=15)**	**(N=15)**	
Males/Females	8/7	7/8	
Age (years)	24.8 ± 8.23	27.67 ± 3.84	N.S.
Body mass index	26.1 ± 5.08	22 ± 3.89	N.S.
TRT (min)	509.73 ± 27.27	433.97 ± 30.87	**<0.001**
TST (min)	462.67 ± 48.78	398.17 ± 39.81	**<0.001**
TWT (min)	47.07 ± 33.84	35.8 ± 16.93	N.S.
SE (%)	90.66 ± 6.78	91.61 ± 4.2	N.S.
WASO (min)	42.5 ± 33.91	28.6 ± 17.01	N.S.
SOL (min)	4.57 ± 5.24	5.37 ± 3.64	N.S.
LS (%)	63.14 ± 8.83	71.47 ± 6.65	**0.009**
DS (%)	11.84 ± 7.13	7.93 ± 4.97	N.S.
REM (%)	25.02 ± 6.13	20.58 ± 5.27	**0.049**
REM latency (min)	25.53 ± 36.19	100.37 ± 54.54	**<0.001**
PLMS (events/h)	4.15 ± 8.14	2.47 ± 4.32	N.S.
AI (events/h)	1.09 ± 1.4	0.13 ± 0.15	**0.016**
HI (events/h)	5.53 ± 7.48	1.06 ± 1.03	**0.035**
In MSLT			
Mean SOL	3.65 ± 3.61	-	
Mean SOREMP	3.33 ± 1.07	-	

TRT = Total recording time; TST = Total sleep time; TWT = Total wake time; SE = Sleep efficiency; WASO = Wake after sleep onset; SOL = Sleep onset latency; LS = Light sleep; DS = Deep sleep (Slow wave sleep, SWS); PLMS = Periodic limb movements during sleep; AI = Apnea index; HI = Hypopnea index; SOREMP = Sleep onset REM period; N.S. = Not significant

2.2. PSG recording and data

All participants underwent nighttime PSG, and the narcolepsy group underwent additional daytime MSLT at the Center of Sleep and Chronobiology of Seoul National University Hospital. Before both the PSG and MSLT, clinicians checked the signals of participants to obtain the reference readings for several situations like breathing, switching body position, eye blinking, and other signal checks. Participants in both groups slept between 7 and 8 hours during the night time for PSG, and the narcolepsy group slept an additional 5 times for 20 minutes during the day for MSLT. During PSG and MSLT, we collected 20 signals via electrodes and sensors. These included an accelerometer for body position, six EEG from the right and left of the frontal, central, and occipital lobes; two electrooculograms (EOGs) from the left and right eyes, three electromyograms (EMGs) from the left and right tibialis anterior and submental muscles, an electrocardiogram (ECG) from the heart, oxygen saturation from pulse oximetry, thoracic and abdominal movement from a piezoelectric sensor, nasal-oral airflow temperature and pressure. All signals were sampled at 500 Hz using the NEUVO system (Compumedics Ltd., Victoria, Australia) for PSG records, and each sleep stage was scored in 30-second epochs. Sleep stages were scored by trained PSG technologists based on the AASM manual **[34]**, and two clinicians checked the results and diagnosed the participants. We converted five sleep stages (N1, N2, N3, REM, and WAKE) into four sleep stages: light sleep (LS), deep sleep (DS), REM, and WAKE by grouping N1 and N2 as LS, and N3 as DS. Based on the different characteristics of patients with narcolepsy regarding brain activity, body movement, eye movement, and cardiopulmonary function **[5]**, we used EEGs from the brain, EOGs from the eye, EMGs from the leg

and facial parts, and ECGs of heart and respiration information to evaluate their connectivity.

2.3. Data processing

From the selected signals, we derived nine features for further analysis using the TDS. These included heart rate from the ECG (HR), movement power from the chin and leg EMG (CHIN, LEG), respiration rate from the pressure transducer airflow (RESP), eye movement power from the EOG (EYE), and four spectral powers of delta (δ), theta (θ), alpha (α), and beta (β) from the EEG.

Before we applied the TDS method, feature signals were derived or calculated for each of the 1-s windows, and the window was shifted by 0.5 s. Thus, nine feature signals were derived for every 0.5 s to represent temporal variation of their connectivity with maximal temporal detail **[36]**.

EEG: From the central lobe channel of the EEG, we performed frequency analysis and obtained the periodogram for the spectral range of delta (0.5~3.5 Hz), theta (3.5~7 Hz), alpha (7–13 Hz), and beta (13-20 Hz). We chose to use the central lobe's EEG signal as we hypothesized that this difference would come from the difference in characteristics of the motor control sections between the narcolepsy patients and controls and the motor cortex, which controls the motor control area, is located in the central lobe. Moreover, we confirmed that there were no significant differences according to brain areas in our study. EEG brain waves were obtained in a 1-s window (500 Hz), and the processing window was shifted by 0.5 s in every iteration.

ECG: ECG R-peaks from QRS waves were detected with a peak detection algorithm and double-checked after automated processing of the algorithm. Heart rates were obtained with a 2-s window and a 1-s shift process, as the instantaneous heart rate could be calculated by the interval of successive heartbeats, and at least two beats

were needed to calculate heartbeats. After obtaining the heart rates, we resampled them to satisfy the 1-s window with a 0.5-s shift.

Respiration: Usual respiration rates are approximately 12-20/min in adolescents and adults. This means that one respiration cycle requires at least 5-6 s. It is not possible to obtain respiration rates with a 1-s window. Therefore, we derived the respiration rates using 5-s, 10-s, 15-s, and 30-s windows and resampled them to a 1-s window. Respiration rates for different windows were calculated using the auto-correlation method from a pressure transducer airflow sensor with a filter range of 0.15 to 0.5 Hz, and they were compared by correlation with the reference respiration rate derived from a 30-sec window. Pearson's correlation coefficient was adopted for 5-s, 10-s, and 15-s windows, and each showed 0.653±0.08, 0.535±0.11, 0.381±0.17 in patients with narcolepsy and 0.676±0.11, 0.57±0.13, 0.379±0.16 in controls respectively. As a 5-s window lost a large amount of information, we chose to use a 10-s window and resampled it into a 1-s window with a 0.5-s shift.

EMG and EOG: EMG information from the facial submentalis muscle (CHIN) and tibialis anterior muscle (LEG) and EOG information from bilateral eyes (EYE) were used to obtain movement information in normalized form, and the square root of the average of the squared data from the absolute value of the signal was extracted every iteration with a 1-s window and a 0.5-s shift.

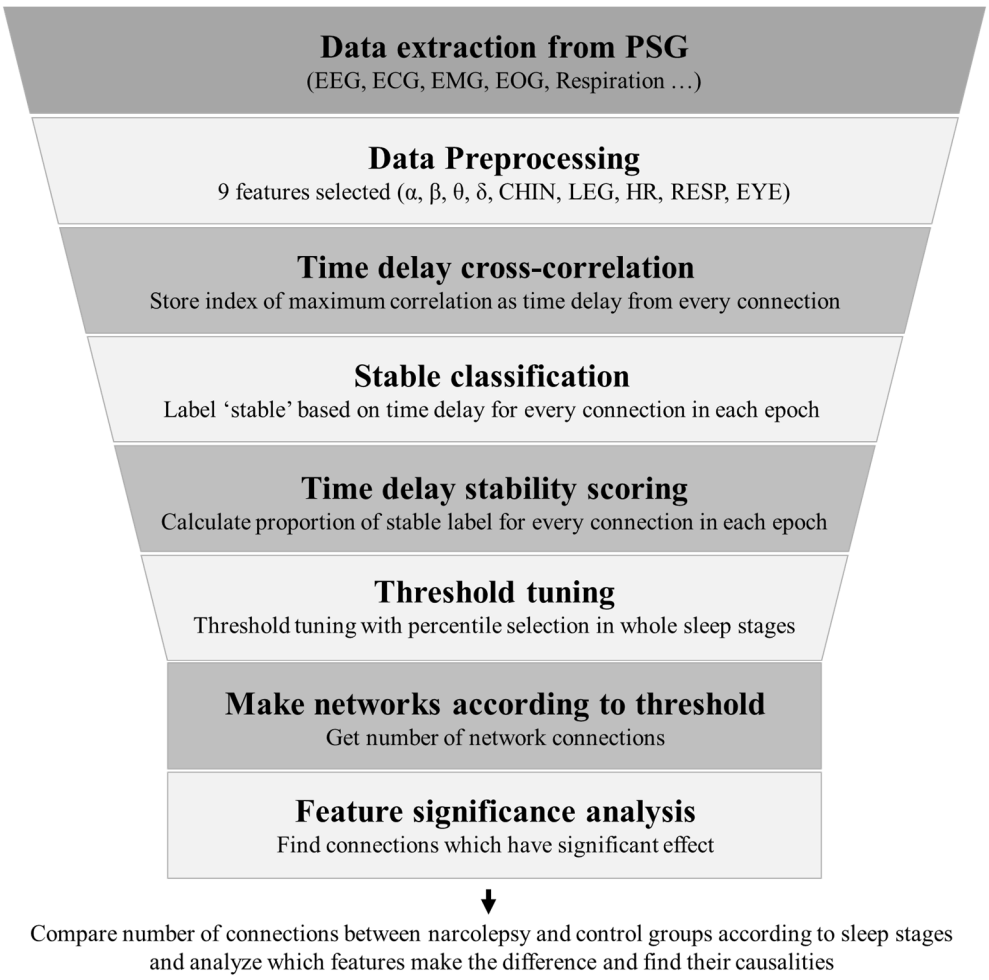

Figure 2-1. Overall pipeline flowchart that shows the entire procedure to analyze network connectivity in the narcolepsy and control groups.

2.4. Time delay cross-correlation

To analyze the transition between sleep stages and determine the effect of physiological connectivity in greater temporal resolution, one epoch (30-s signal) was shifted by 15-s to create a 30-s window with 15-s shift for every iteration. As the second shift was not compatible with the number of sleep stages from 30-s epochs (double the number of sleep stages), epochs that shared the latter 15 s of the previous stage and the first half of the latter part of the sleep stage were labeled using the score of the previous stage **(Figure 2-3)**. Each sample of processed sleep-feature data represented 1 s of their connectivity information with 0.5-s time resolution. This means one epoch consisted of 60 sample points. To obtain the connectivity information of an epoch, cross-correlation was conducted for all nine biosignals. As nine features were selected, there were 36 cases of cross-correlation (Complete graph with 9 points; ${}_9C_2 = 36$, **Figure 2-2**).

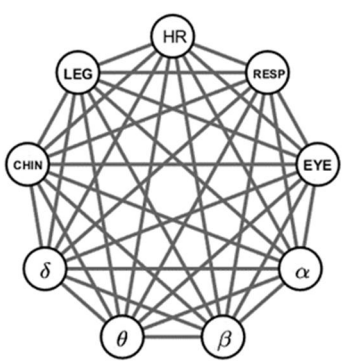

Figure 2-2. Complete graph with 9 points could make 36 connection.

Data within one epoch were normalized to zero mean and unit standard deviation to maintain constant trends throughout the data. After normalization, cross-correlation was performed. Cross-correlation revealed the scores of the correlation coefficient between two data with a sliding dot product method, that is, by shifting data within a window **(2-1)**. We found the time index that had the maximum absolute value of the correlation score to consider positive and negative correlations. We nominated the time index with the maximum value of cross-correlation as the time delay of the corresponding epoch **(2-2)**.

$$\gamma_{XY}[t] = \frac{\sum_i^n (X_i - \bar{X})(Y_i[t] - \overline{Y[t]})}{\sqrt{\sum_i^n (X_i - \bar{X})^2} \sqrt{\sum_i^n \left(Y_i[t] - \overline{Y[t]}\right)^2}} \quad , -n < t < n. \qquad \textbf{(2-1)}$$

$$\tau = argmax\{\gamma_{XY}[t]\} \qquad \textbf{(2-2)}$$

where, X, Y = signals to compare; Y[t] = time shifted signal with t; n = length of signals; $\gamma_{XY}[t]$ = time shifted Pearson's correlation coefficient; τ = time delay index.

In short, we used time delay cross-correlation to determine the time delay between two signals by moving the data and finding the maximum score of the correlation coefficient in each epoch. We restricted the range to find the maximum time delay index in a small time area from -5 to 5 s to finely investigate physiological changes among the biosignals of each epoch **(Figure 2-4)**. Indices of time delay were stored and used to calculate the time delay stability for whole biosignal combinations depending on the sleep stages.

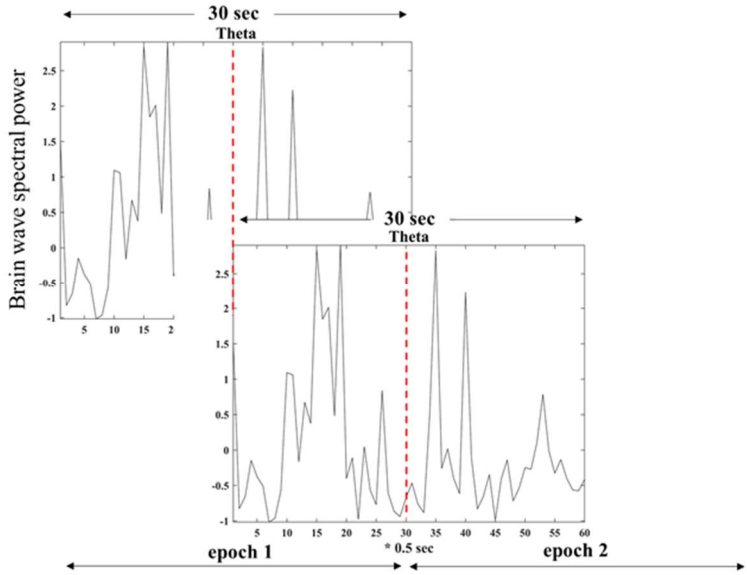

Figure 2-3. Thirty-second window shifted by 15 seconds with overlapping

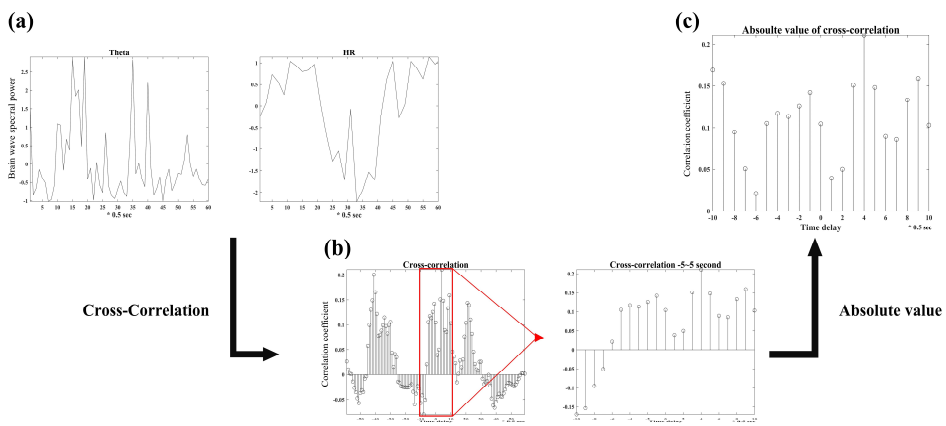

Figure 2-4. Example of time delay index acquisition between two signals

Theta brain wave and heart rate (HR) signals of one epoch are extracted from one participant, and the time delay method is applied. (a) Extracted information is shown from one epoch of each signal, theta EEG band power and HR. (b) After applying cross-correlation between two signals, correlation-coefficient values are obtained depending on the time delay for the restrict area from -5 to 5 seconds. (c) The absolute value is applied to the result of cross-correlation within the restricted window and time delay index, which represent the highest correlation-coefficient is stored.

2.5. TDS methods

Time delay stability (TDS) can quantify interactions among biosignals by considering temporal variations in physiological networks and connectivity within the body. TDS can show how people maintain their states stably and how strongly the connections are between organs. In addition, it can reveal the synchronization of signals by comparing the correlation scores using time information within an epoch. This allows us to identify the complex networks of body interactions and understand systemic connectivity. To calculate the TDS, the time delay index for each epoch was loaded with the scored sleep stage. Each epoch had 36 time delays for all possible combinations of the two features. We classified as "stable" each network of signals containing segments with a length of five successive sleep epochs. Based on time delay of the first epoch in segment (τ), if more than four epochs from the segment had time delay indices within the range of τ-1 to τ+1 s ([τ-1, τ+1]), then the first epoch was labeled "stable". This procedure was repeated with a five-epoch window and a unit epoch shift. All 36 networks had their own "stable" classification using the above criteria and were packed within each epoch. This means that every sleep epoch had 36 networks, each labeled as stable or not stable **(Figure 2-5)**.

We quantified stability of the networks and analyzed them for each of four sleep stages. The TDS score was obtained by calculating a proportion of "stable" classifications for each sleep stage during whole sleep. Every sleep stage (WAKE, REM, LS, DS) received a TDS score by dividing the number of "stable" classifications by the number of each sleep stage for every network.

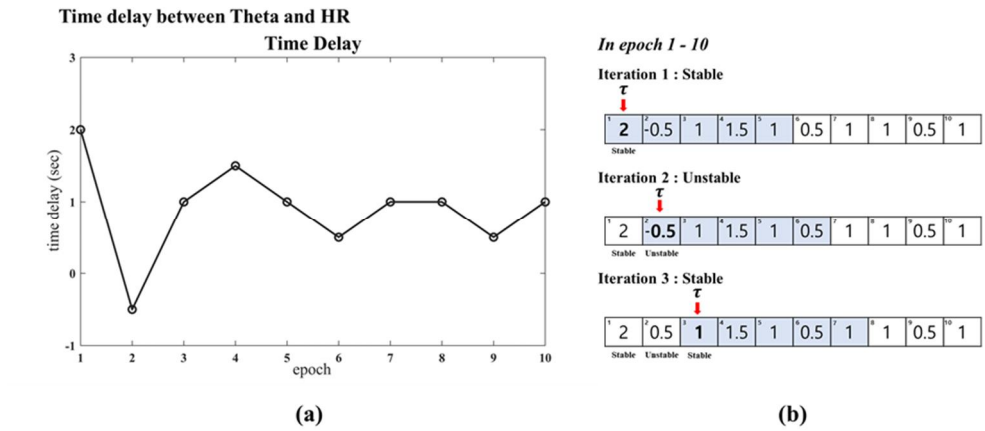

Figure 2-5. The above figure shows an example of "stable" classification of an epoch for the connectivity of theta and HR signals. (a) Time delay indices of epochs are used to classify "stable." (b) From epoch one to ten, the labeling procedure is conducted based on the time delay index of the first epoch in segments (τ). We check whether the segments are stable for every epoch.

2.6. Threshold tuning

In previous research, TDS was used to create links between two signals. After the threshold value was obtained, if the TDS score of each network was over the threshold, two signals were connected by a link, and combinations of these connections created a visualized topology. It showed a complex physiological system depending on the sleep stages with quantified and visualized modalities **[8]**. However, as there are many conditions and differences among an individual's physiological states, to minimize individual variations, we normalize the results of TDS according to the participants. We derived the optimal threshold to build links between the signals for each sleep stage. To apply the common threshold value to the four sleep stages, the score distribution of the whole TDS without classification of sleep stages was used to derive the threshold. The average TDS scores of 36 networks were sorted without consideration of sleep stages, and the lower percentile was selected as the possible threshold. The lower 10, 20, 30, 35, and 40 percentiles were selected to find the optimal threshold and compare their performances in differentiating narcolepsy from the controls. These thresholds, without consideration of sleep stages, were applied to each sleep stage. If the TDS of a network was over the threshold, a link was built between two signals. This made network connections for each sleep stage, showed how the connections were comprised, and revealed how the changes developed according to sleep stage. By summing the number of linked connections of each sleep stage, we obtained the number of network connections and compared it among sleep stages and between the two groups **(Figure 2-6)**.

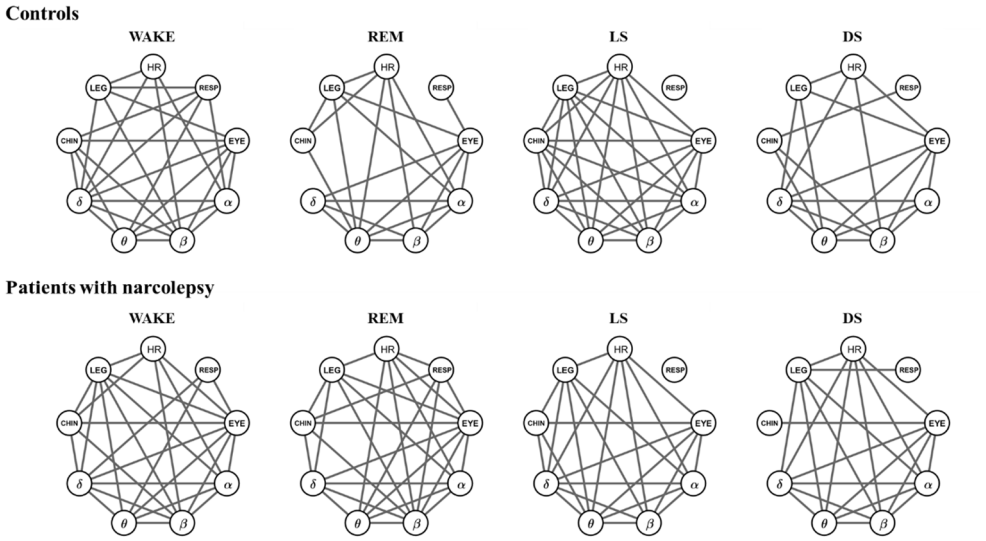

Figure 2-6. Above are examples of network connections in patients with narcolepsy and controls when a 30th percentile threshold is applied. Patients with narcolepsy and controls have different connections, which are visualized as topologies.

The lower percentiles were fine-tuned to obtain the optimal threshold, and we compared the number of connections. In previous research, healthy controls showed a higher number of connections in the WAKE and LS stages, while the REM and DS stages had fewer connections. When comparing sleep stages between the two groups, there were significant differences between REM/DS and WAKE/LS (t-test $p < 0.001$) in each group **[8]**. Based on this, we changed the percentile for the threshold and compared the number of connections in the groups. As a result, the threshold with the lower 30th percentile showed the maximal difference and satisfied those conditions. P-values revealed significant differences when we compared WAKE with REM and DS ($p < 0.001$) and also when we compared LS with REM and DS ($p < 0.05$, $p < 0.01$); these differences are similar to previously reported results **[8]**.

If the threshold exceeded the 30th percentile, it began to lose the network connections information. A high threshold made it difficult to build links using the TDS method. In the 35th percentile, there was no significant difference between REM and LS. The higher the threshold used, the more significant the difference; the information was eliminated so that we could not distinguish and compare the differences among sleep stages. Additionally, when comparing patients with narcolepsy and controls, there were significant differences in the number of network connections during REM for all percentile thresholds except for 35th. In the 30th percentile, we observed the largest significant difference (p-value of 10th: 0.031, 20th: 0.042, 30th: 0.022, 35th: 0.108, 40th: 0.047) **(Figure 2-7)**. From these results, we chose to use the lower 30th percentile to compare network connections and analyze network connectivity between the groups.

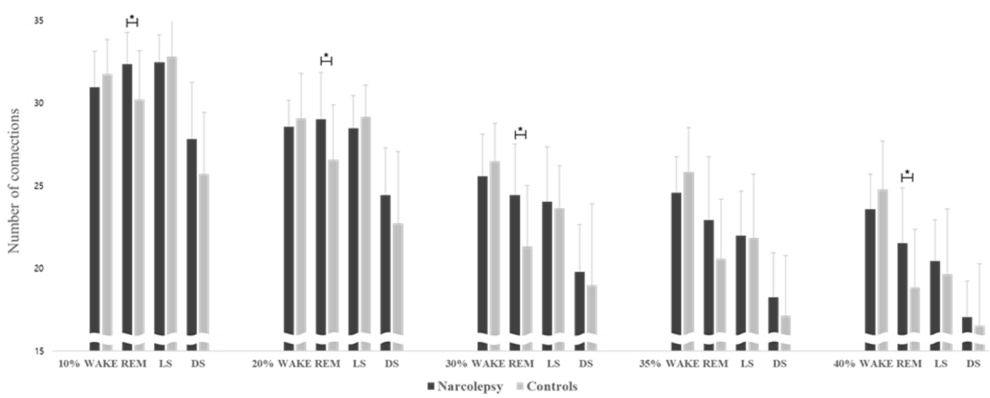

Figure 2-7. Number of connections in different sleep stages for different percentile thresholds (* $p < 0.05$).

2.7. Test-retest reproducibility

We could show the reliability and reproducibility of our method by using the data from the MSLT (Multiple sleep latency test) which was done after the nighttime PSG in patients with narcolepsy. Since the MSLT was repeated 5 times, for 20 minutes during daytime sleep, and each segment of the MSLT used the same process as the PSG analysis, we could investigate the same participant 5 times to simulate the test-retest procedure. However, since the MSLT has a short sleep time compared to nighttime PSG, MSLT seldom has deep sleep. Since MSLT also could not show enough light or REM sleep due to the short duration, we excluded any data which could not make over 10 network connections. In patients with narcolepsy, we conducted same procedures to get the number of network connections and we compared distributions of five MSLT sleeps with analysis of variance (ANOVA).

2.8. Brain and peripheral connections

As we used nine features to create a physiological network (**Figure 2-6**) and a connection was made between two features, there was a total of 36 connections. We classified features as brain or peripheral areas. The brain areas included delta (δ), theta (θ), alpha (α), and beta (β) features, which were classified using EEG data; the other five features were classified as peripheral parts of the body. To analyze and compare the number of connections between and within the brain and peripheral areas, we defined three types of connections: brain-brain connections, brain-periphery connections and periphery-periphery connections. We analyzed the number of network connections not only for whole networks but also within and between separated body parts.

2.9. Effect of brain-brain connections according to brain areas

EEG signals in our study were measured from three brain areas: the central, frontal, and occipital lobes. We chose to use the central lobe's EEG signal as we hypothesized that the difference between narcolepsy patients and controls would come from the different characteristics of the cortex's motor control regions and the motor cortex, which controls the motion area, is located in the central lobe.

As EEG signals from different brain areas share similar trends, we hypothesized there would no difference according to brain areas. However, to confirm that there are no significant difference and to determine if there would be any reasonable factors, we did additional analysis where we separated the signal by extracting signals from different parts of the brain areas. We applied the same algorithm we did during our assessment of the central lobe to three different parts of the brain areas: frontal, occipital and central lobes, and analyzed their relationship.

2.10. Feature significance analysis

As the narcolepsy and control groups showed differences in network connectivity during nighttime sleep, there must be physiological distinctions in the features according to sleep stages. To identify which feature connections differed and influenced the number of network connections between the narcolepsy and control groups, we conducted feature significance analysis by comparing TDS values for whole connections among features of the two groups. The same threshold tuning and normalization was conducted for each participant, as individuals have different physiological characteristics and the variations may cause disturbance in the analysis. Whole TDS values were normalized for each participant using the proportion method. Thirty-six connections each had their own TDS value, and each connection was normalized using the summation of whole TDS values **(2-3)**.

$$N_{th}\ \text{Normalized TDS} = \frac{\text{TDS}(\text{connection}_N)}{\sum_{n=1}^{36}[TDS(connection_n)]} \times 100 \qquad \textbf{(2-3)}$$

Normalized TDS values for patients with narcolepsy and controls were compared according to sleep stage. We sought to determine which components contributed to the difference in network connections between the two groups by determining connections with significant differences.

2.11. Network directionality with correlation

After analyzing the feature significance of each connection, we also compared the network directionality by considering both the negative and positive correlations from the time delay cross-correlation. As described in section **2.4**, we determined the time delay index from the absolute value of correlation, or mixed correlation [**37**]. However, by analyzing each positive and negative correlation, we could find the directionality of each significant connection, which was helpful in interpreting the difference in the characteristics between the patients with narcolepsy and controls more specifically. Without applying the absolute value in time delay cross-correlation, we extracted positive correlation values from the time delay cross-correlation by selecting the maximum score with stored time delay index. Likewise, negative correlation values were extracted from the minimum score of cross-correlation with time delay index. We analyzed the connections' correlation which were classified as 'stable' from the mixed correlation and compared both the positive and negative correlations according to time delay and sleep stages between the two groups.

2.12. Verifications of network connectivity as classifier

To verify that the network connectivity could be a potential biomarker to distinguish patients with narcolepsy and controls, it should show better performance as a classifier than unimodal feature's performance from PSG. Although many studies have tried to determine the unimodal difference between narcolepsy and controls, their findings are inconsistent. Except for HRV and brain waves, the features we used are not well studied. Furthermore, EMG as a biomarker only showed any difference in previous studies when the RSWA was calculated from it **[16]**. Based on these, we hypothesized that interactions among biosignals with network connectivity could determine other explanations for the difference which could help us to access causal relationships, with further research.

We extracted each data from the 9 unimodal biosignals and compared their effect to distinguish narcolepsy and control groups during REM sleep. Furthermore, we compared the number of network connections in both groups as classifier with each of unimodal feature. We classified patients with narcolepsy and controls based on the average number of narcolepsy features during REM sleep. In other words, we classified narcolepsy and control groups during REM sleep using the average number of each narcolepsy feature as threshold. After all, we evaluated their performance with sensitivity, specificity, and accuracy to distinguish both groups.

2.13. Classification with support vector machine

To verify if network connectivity with the TDS method could be a potential biomarker, we conducted a simple classification between narcolepsy and control groups. We extracted two features: the number of network connections and merged TDS values from the significant connections affecting the number of connections from section **2.10**. The merged TDS was obtained by summing each feature's TDS value with a constant weight **(2-4)**.

$$\text{Features' TDS} = \sum_{i=1}^{N}(W \times significant\ \ feature'\ s\ \ TDS_i) \qquad \textbf{(2-4)}$$

where, N= number of significant features, W= constant weight.

With the Features' TDS and the number of connections, we scattered their distributions and classified them with a support vector machine (SVM), which finds the best decision boundary to distinguish two groups by determining the maximum margin between each nearest group's data point. Margins represent the distance between the decision boundary to support vectors, which were used to guide finding the best decision boundary and usually located near to it. Thereafter, based on the decision boundary from SVM, we investigated their performance as a classifier by calculating the sensitivity, specificity, and accuracy.

3

Results and Discussion

3.1. Results

3.1.1. Network connections between narcolepsy and control groups

After we applied the TDS method with a 30th percentile threshold, we found a difference in the overall number of network connections during sleep. In the control group, sleep stages showed the same trend as in previous research, with a higher number of connections in WAKE and LS, and a lower number in REM and DS **[8]**. However, although they showed a trend similar to that of the controls, the narcolepsy group showed a significantly higher number of connections in REM sleep ($p = 0.022$). The narcolepsy group showed about three more connections (narcolepsy: 24.47 ± 2.87, control: 21.34 ± 2.65) compared to control group **(Figure 3-1)**.

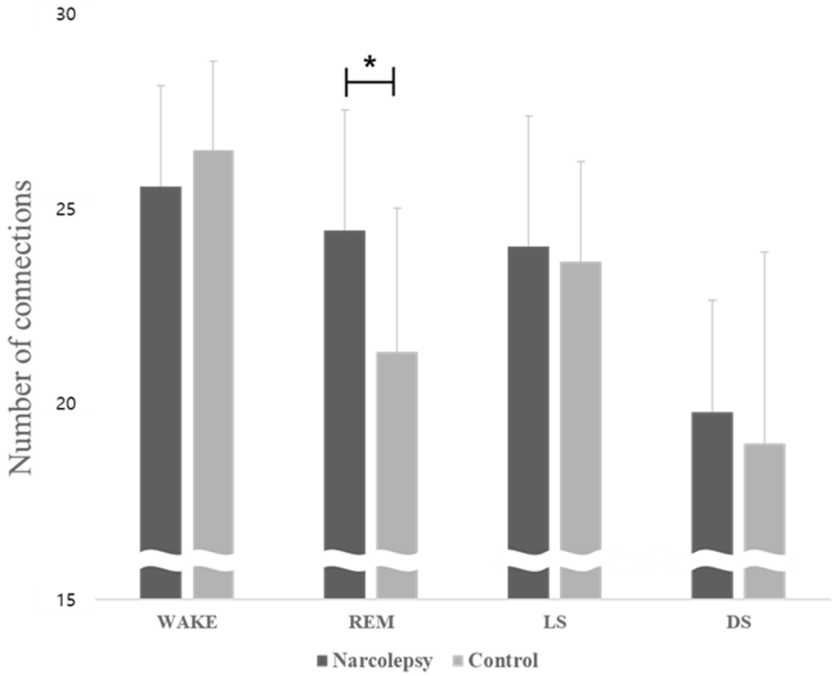

Figure 3-1. Network connections between narcolepsy and control groups (* $p < 0.05$).

For a more detailed analysis, we divide this whole network into three parts (brain-brain, brain-periphery, and periphery-periphery) and compared the number of connections between narcolepsy and control groups. As brain lobes are highly connected and four areas have high correlation, almost every connection between brain signals was over the threshold. As a result, brain-brain connections were fully connected to each other; they did not affect the whole number of connections. Meanwhile, the brain-periphery and periphery-periphery areas showed fluctuations among sleep stages and between the two groups. As the difference between groups was shown in whole connections of REM sleep, we focused on the REM sleep stage. Brain-periphery networks in REM sleep showed a higher number of connections in patients with narcolepsy compared to controls, but the difference was not significant

(p = 0.135). However, periphery-periphery networks also showed a higher number of connections in patients with narcolepsy than controls, and the difference was significant in REM sleep (p = 0.0017). This shows that the overall number of connections is more likely to be affected by brain-periphery and especially periphery-periphery areas of physiological interactions in the body **(Figure 3-2)**.

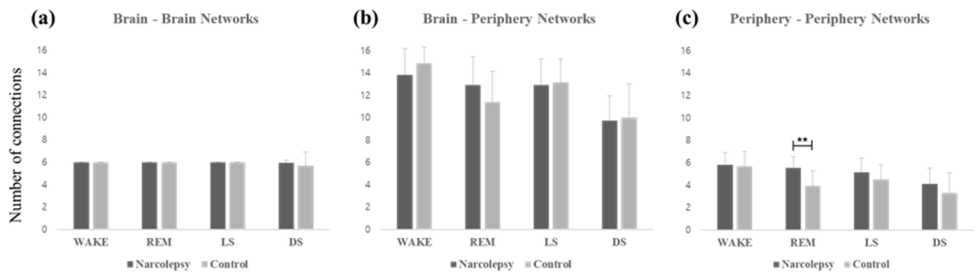

Figure 3-2. Brain-brain network connections (a) do not show differences among sleep stages or between the two groups. However, brain-periphery network (b) and periphery-periphery network (c) connections have fluctuations, and the narcolepsy group shows significantly higher connections than controls in periphery-periphery REM sleep (** $p < 0.01$).

3.1.2. Test-retest analysis for reproducibility

With data from daytime sleeps after the night time PSG of patients with narcolepsy, we could retest our TDS based connectivity analysis multiple times to ensure that our method has reproducibility. **Table 3-1** shows the results of network connections in MSLT which we compared the distribution of to determine whether there was any difference between with ANOVA analysis. The F- and P-value scores indicate that there is no significant difference among MSLT in every sleep state. Although MSLT has different characteristics from nighttime PSG and therefore has limitations to definitively show the test-retest reliability of PSG, this multiple verification of MSLT in the same participants could be an indication of our methods' reproducibility.

Table 3-1. Distribution of number of network connections in MSLT sleep with ANOVA analysis

MSLT1			MSLT2			MSLT3		
WAKE	**REM**	**LS**	**WAKE**	**REM**	**LS**	**WAKE**	**REM**	**LS**
22.78 ± 4.73	25.92 ± 6.49	19.56 ± 3.65	21.38 ± 4.97	24.6 ± 4.34	21.45 ± 5.69	20 ± 4.47	25.83 ± 5.15	21.62 ± 6.05

MSLT4			MSLT5			ANOVA	*F*	*P*
WAKE	**REM**	**LS**	**WAKE**	**REM**	**LS**	**WAKE**	0.449	0.772
21.8 ± 5.47	24.58 ± 4.07	20.27 ± 3.96	22.8 ± 4.66	26.17 ± 5.81	20 ± 3.58	**REM**	0.194	0.941
						LS	0.309	0.871

3.1.3. Significant feature identification

To identify which components in a network affect the total number of connections, we conducted a feature significance analysis. As the number of connections differed in REM sleep, we focused on REM sleep connections and visualized them using topology **(Figure 3-3)**. Significant differences were observed between HR and theta (θ) brainwaves ($p = 0.05$), HR and eye movement ($p = 0.026$), and chin movement and respiration rate ($p = 0.043$), and their values were higher in patients with narcolepsy than in controls. Delta (δ) and alpha (α) brainwaves ($p = 0.0072$) and delta (δ) and theta (θ) brainwaves ($p = 0.03$) also showed significant differences, but they were higher in controls than in patients with narcolepsy. These components may affect overall network connections; however, as brain-brain networks usually exceed the thresholds, they do not affect the number of connections **(Figure 3-2)**. Therefore, we excluded brain-brain networks to identify the components significantly affecting the number of connections **(Figure 3-3-b)**.

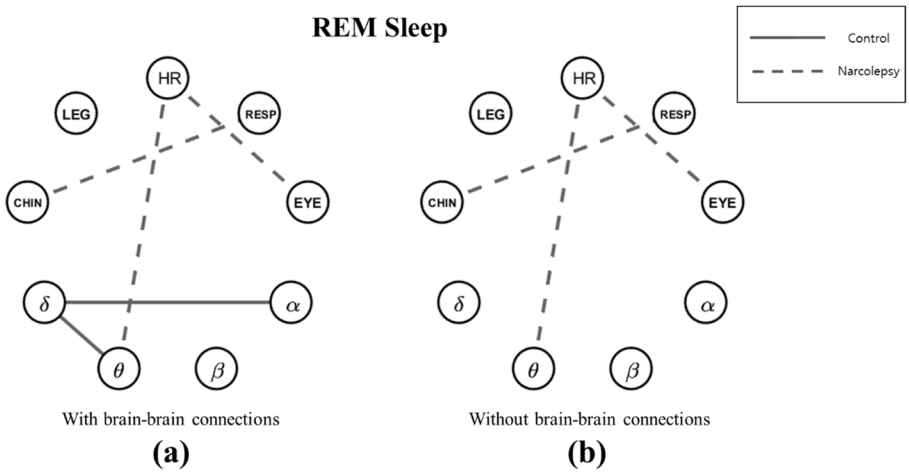

Figure 3-3. Network topologies of biosignals that appear significant difference. A dotted line represents a connection with a significantly higher TDS in the narcolepsy group than in the control group ($p < 0.05$), and a solid line represents a connection with a significantly higher TDS in the control group than in the narcolepsy group. Brain-brain connections are shown in (a), but as brain-brain connections do not affect the total number of network connections, they are eliminated in (b).

Brain-periphery and periphery-periphery networks mainly affected the number of connections among the 36 cases. The overall feature significances in REM sleep are expressed with p-values and differences in connectivity in **Figure 3-4**. These values show how the normalized TDS values of the connections differ between the two groups. Although the brain-brain area reveals significant differences in α-δ and θ-δ connections, it does not affect networks; brain-brain connectivity was stronger in controls than in patients with narcolepsy. On the other hand, the brain-periphery and periphery-periphery areas usually show higher connectivity in patients with narcolepsy, especially in θ-HR, HR-EYE, and CHIN-RESP connections.

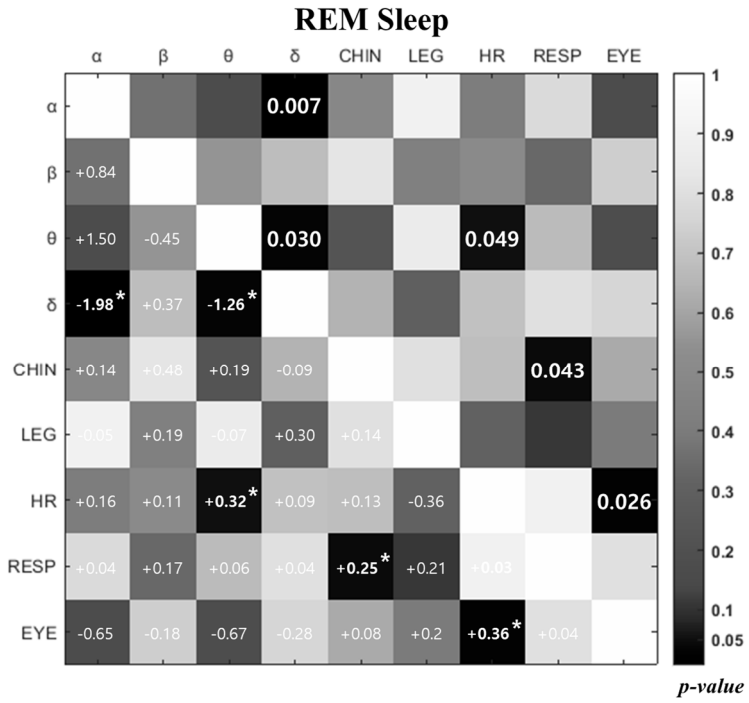

Figure 3-4. The matrix above shows the differences in feature connectivity and their p-values in REM sleep. The number on upper part of the diagonal matrix shows significantly different connections with p-values; the number on lower part of the matrix shows the differences in normalized TDS values between the narcolepsy and control groups (* $p < 0.05$). The narcolepsy group values are subtracted from the control group values; positive numbers indicate the narcolepsy group has higher connectivity than the control group, and negative numbers indicate the control group has higher connectivity than the narcolepsy group. The connections with α-δ, θ-δ, θ-HR, CHIN-RESP, and HR-EYE reveal significant differences and the matrix shows how they are distributed between the narcolepsy and control groups during REM sleep.

3.1.4. Effect of brain-brain connections according to brain areas

Even though we measured the EEG of the brain areas in different locations, they had a high correlation-coefficient when their signals were compared. Because of the high correlation, they could not make a difference in network connection as they mostly exceeded the threshold (**3.1.1, 3.1.3**, **Figure 3-2**). However, to determine if there are any meaningful information, we applied our network connectivity methods with changed brain area. To confirm that there are no noteworthy differences in connections with brain and peripheral areas, we compared distributions of network connections during sleep in both of narcolepsy and control groups (**Table 3-2**).

The number of network connections does not show any significant difference when compared to the results obtained from the central lobe, which we initially dealt with in the previous result. The distributions were also similar in both the narcolepsy and control groups. Other than the frontal lobe showing a marginally significant difference (p = 0.07) between the two groups, no other results show significant difference, and the central lobe during REM sleep showed the most meaningful significant difference. Based on the results of the analysis of the other parts, we confirmed that they do not show any significant difference among them.

Table 3-2. Number of network connections according to brain areas

	Central lobe				Frontal lobe				Occipital lobe			
	WAKE	**REM**	**LS**	**DS**	**WAKE**	**REM**	**LS**	**DS**	**WAKE**	**REM**	**LS**	**DS**
Control	26 ± 2.19	21.34 ± 3.49	24.33 ± 2.65	18.73 ± 5.51	25.6 ± 2.82	22 ± 4.03	24.47 ± 2.75	19.13 ± 3.79	26.47 ± 2.63	22.13 ± 3.54	23.87 ± 3.42	19.47 ± 3.91
Narcolepsy	25.87 ± 2.28	24.47 ± 2.87	23.93 ± 2.67	19.8 ± 2.95	25.53 ± 2.21	24.4 ± 2.55	24.47 ± 2.75	20.07 ± 3.04	25.33 ± 1.53	24.73 ± 2.98	23.73 ± 2.84	20.13 ± 3.88
p-value	0.876	**0.022***	0.694	0.528	0.783	**0.07**	0.944	0.479	0.175	**0.044***	0.911	0.654

For detailed explanations, we analyzed the next process of significance analysis with every brain EEG location. In the central lobe, we could get five significant connections; α-δ, θ-δ, θ-HR, HR-EYE, CHIN-RESP during REM sleep (**Figure 3-5-b**). In the frontal and occipital lobes, they also showed similar network of significant features. Compared to the central lobe, the frontal lobe lost the HR-EYE connection, and the occipital lobe lost the θ-δ connection. However, even though they could not reach a significant difference ($p < 0.05$), they were marginally significant (HR-EYE in frontal lobe p = 0.062, θ-δ in occipital lobe p = 0.056) on their connections. Based on these results, we could find no remarkable differences among three different brain areas in brain-periphery and periphery-periphery connections.

We considered that the loss of HR-EYE in the frontal lobe is due to relatively low frontal lobe activation as the visual function is mainly located in the occipital lobe. With respect to the location, the central lobe could show both frontal and occipital characteristics.

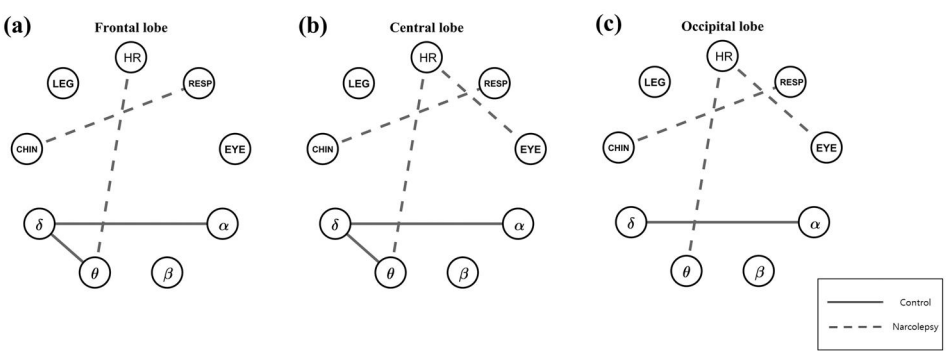

Figure 3-5. Connections of significant features from different brain area.

In addition, under the assumption of possible meaningful brain-brain connections among the three brain areas, we selected brain waves from different parts of the brain lobes and analyzed their relationship. In **Figure 3-5**, there were two meaningful connections: α-δ, θ-δ and a marginal significance in the occipital lobe. When we created a feature network, all features except θ and δ in the occipital lobe had connections between α-δ and θ-δ. It means, that not only α-δ and θ-δ themselves are in the same brain area, but also between other brain area's features. For example, central lobe's alpha (α) made connections with frontal lobe's delta (δ) and occipital lobe's delta (δ). Even though the occipital lobe could not make connections, they also showed marginal significances that were close to make connections ($p < 0.08$). **Figure 3-6** shows network connections as topology and **Figure 3-7** describes their connections on the brain.

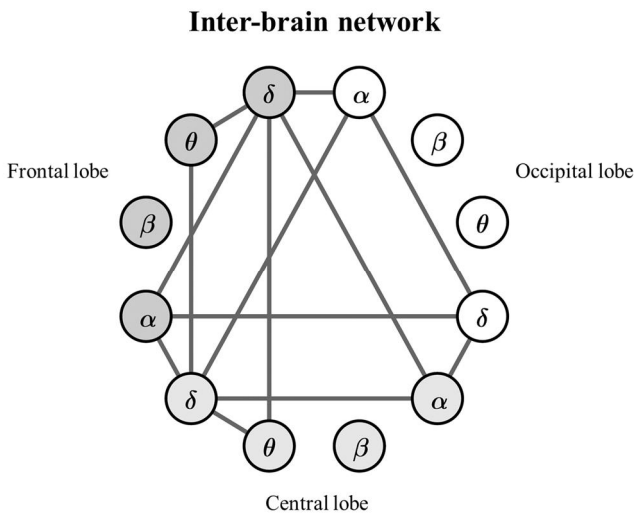

Figure 3-6. Network connections of brain waves among three difference brain areas shown as topology.

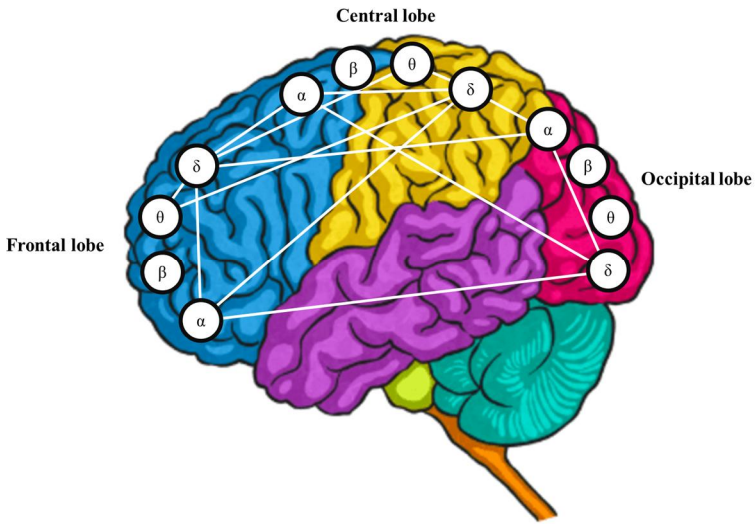

Figure 3-7. Network connections of brain waves among three different brain areas displayed over a brain scheme.

***Brain image from:** https://www.twinkl.es/teaching-wiki/brain-for-kids*

These analysis results indicate a delicate difference of connectivity according to locations in brain lobes. As they are highly synchronized and have similar waveform trends, the differences from the various brain areas may have a small effect on network connections.

Therefore, based on the result of the similar trends and that central lobes showed the highest significance, we decided to use central lobe's EEG, as it could include both frontal and occipital characteristics with the benefit of a central location.

3.1.5. Network directionality with correlation

Three connections (θ-HR, HR-EYE, CHIN-RESP) showed a significant effect on overall network connectivity from the significant feature identification. For further explanations of these connections, we considered directionality by analyzing the positive and negative correlation. From time delay cross-correlation, we extracted time delay indices and correlation values from stable epochs of three connections during REM sleep (**Figure 3-8**). From the θ-HR connection, as it showed superiority in positive side, we extracted a positive correlation for both groups; however, patients with narcolepsy showed a significantly higher positive correlation for the average value of all time delay indices (-5s ~ 5s, **Figure 2-4-b**) when compared to controls (Narcolepsy: 0.382 ± 0.118, Controls: 0.317 ± 0.122, p = 0.05). From the HR-EYE and CHIN-RESP connections, as they showed superiority in the negative side, we extracted negative correlations for both groups; however, patients with narcolepsy showed significantly higher negative correlations for the average values of all time delay indices when compared to controls (HR-EYE; Narcolepsy: -0.459 ± 0.126, Controls: -0.353 ± 0.101, p = 0.023, CHIN-RESP; Narcolepsy: -0.509 ± 0.092, Controls: -0.421 ± 0.095, p = 0.018).

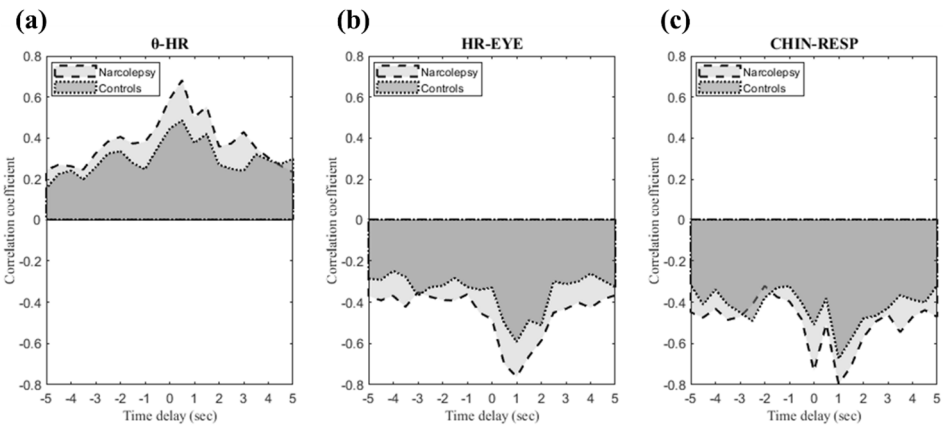

Figure 3-8. Feature connections' directionality based on the correlation and time delay. Three connections which showed significant effect to network connections during REM sleep between narcolepsy and control groups were analyzed according to the correlations' direction and time delay indices from -5s to 5s. The connections showed trends of distribution between two signals according to time delay. (a) θ-HR with positive correlation and θ precedes HR, (b) HR-EYE with negative correlation and HR precedes EYE, (c) CHIN-RESP with negative correlation and CHIN precedes RESP. Compared to controls, patients with narcolepsy showed higher levels of correlation for both sides in all the three connections' correlation.

3.1.6. Performance comparison between unimodal biosignal and connectivity

To compare the performance of connectivity and unimodal features between the narcolepsy and control groups, we extracted each signal from the 9 biophysical features we used in our study and analyzed them (**Table 3-3**). We compared each feature between the narcolepsy and control groups, and found that the only significant difference among all sleep stages was the theta brain wave **[15,49,50]**. We also found marginally significant differences in the HR **[53]**.

Table 3-3. Unimodal differences between patients with narcolepsy and controls in 9 features of biosignals

	Alpha				Beta				Theta			
	WAKE	REM	LS	DS	WAKE	REM	LS	DS	WAKE	REM	LS	DS
Narcolepsy	0.151 ± 0.08	0.116 ± 0.05	0.09 ± 0.03	0.04 ± 0.01	0.08 ± 0.06	0.1 ± 0.19	0.09 ± 0.19	0.06 ± 0.17	0.11 ± 0.03	0.17 ± 0.06	0.13 ± 0.04	0.07 ± 0.03
Controls	0.12 ± 0.06	0.1 ± 0.04	0.09 ± 0.04	0.04 ± 0.04	0.14 ± 0.08	0.16 ± 0.15	0.12 ± 0.11	0.05 ± 0.09	0.08 ± 0.03	0.12 ± 0.03	0.1 ± 0.02	0.06 ± 0.01
p-value	0.28	0.356	0.996	0.766	0.055	0.371	0.677	0.91	**0.017***	**0.019***	**0.014***	**0.02***
	Delta				**CHIN**				**LEG**			
	WAKE	REM	LS	DS	WAKE	REM	LS	DS	WAKE	REM	LS	DS
Narcolepsy	0.66 ± 0.1	0.61 ± 0.16	0.68 ± 0.17	0.83 ± 0.17	0.027 ± 0.026	0.01 ± 0.006	0.011 ± 0.007	0.01 ± 0.008	0.58 ± 0.15	0.26 ± 0.12	0.2 ± 0.08	0.15 ± 0.08
Controls	0.66 ± 0.13	0.62 ± 0.15	0.69 ± 0.14	0.85 ± 0.13	0.039 ± 0.045	0.017 ± 0.019	0.015 ± 0.009	0.012 ± 0.007	0.47 ± 0.21	0.29 ± 0.19	0.26 ± 0.12	0.19 ± 0.14
p-value	0.904	0.961	0.858	0.708	0.415	0.279	0.307	0.484	0.608	0.768	0.635	0.509
	HR				**RESP**				**EYE**			
	WAKE	REM	LS	DS	WAKE	REM	LS	DS	WAKE	REM	LS	DS
Narcolepsy	71.84 ± 9.61	68.07 ± 8.09	66.01 ± 8.44	66.4 ± 9.38	16.4 ± 2.12	17.35 ± 1.64	16.03 ± 2.12	16.26 ± 2.41	0.061 ± 0.064	0.04 ± 0.027	0.032 ± 0.026	0.035 ± 0.022
Controls	66.98 ± 4.91	64.43 ± 6.72	61.02 ± 5.88	63.92 ± 7.3	16.24 ± 1.78	17.39 ± 2	15.77 ± 1.9	16.5 ± 1.91	0.047 ± 0.008	0.044 ± 0.012	0.036 ± 0.009	0.021 ± 0.019
p-value	0.093	0.102	0.08	0.441	0.824	0.955	0.729	0.768	0.627	0.908	0.875	0.273

* $p < 0.05$

We performed additional analysis to investigate their performance as a classifier. Based on the results in the **Table 3-3**, we classified patients with narcolepsy and controls based on the average number of narcolepsy features during REM sleep. In other words, we classified narcolepsy and control groups during REM sleep using the average number of each narcolepsy feature as threshold.

As a result, we could calculate the sensitivity, specificity, and accuracy from each unimodal feature. Additionally, we classified both groups based on the average number of network connections during REM sleep (24.47), and we were able to achieve 0.77 sensitivity, 0.71 specificity, and 0.73 accuracy, and described in the **Table 3-4**. Result as classifier in **Table 3-4** showed the highest performance in network connection followed by theta brain waves, which showed significant differences between groups.

Table 3-4. Binary classification between patients with narcolepsy and controls based on the average threshold

	Sensitivity	Specificity	Accuracy
Alpha	0.55	0.53	0.53
Beta	0.53	0.53	0.53
Theta	0.75	0.67	0.7
Delta	0.6	0.55	0.57
CHIN	0.59	0.62	0.6
LEG	0.41	0.54	0.53
HR	0.64	0.58	0.6
RESP	0.5	0.5	0.5
EYE	0.57	0.56	0.57
Network connection	**0.77**	**0.71**	**0.73**

Even though network connection got the highest performance as classifier compared to other unimodal features, it remains insufficient as a potential biomarker.

3.1.7. Classification performance with SVM

With unimodal signals and number of network connections, they could not make noteworthy performance as a classifier to distinguish narcolepsy and control groups during REM sleep. Some network features showed significant power affect the number of network connections: α-δ, θ-δ, θ-HR, CHIN-RESP, HR-EYE during REM sleep (**Figure 3-3**). We took TDS values from each network feature of REM sleep and merged them by summing them with constant weight (**2-4**). After calculating features' TDS, we scattered participants' data with two features: number of connections and features' TDS (**Figure 3-9**). As we stated, brain-brain connections could not affect the number of network connections (**Figure 3-2-a**), we did two types of analysis: with brain-brain (five features) and without brain-brain (three features) connections. To find the best decision boundary to classify the groups, we used an SVM. As a result, when brain-brain connections were included, the sensitivity was 0.88, the specificity 1, and the accuracy 0.93. Without brain-brain connections, it was a 0.93 for sensitivity, specificity, and accuracy, respectively. These results show that the narcolepsy group has higher connectivity in brain-periphery and periphery-periphery connections, but lower in brain-brain connections in comparison to the control group during REM sleep.

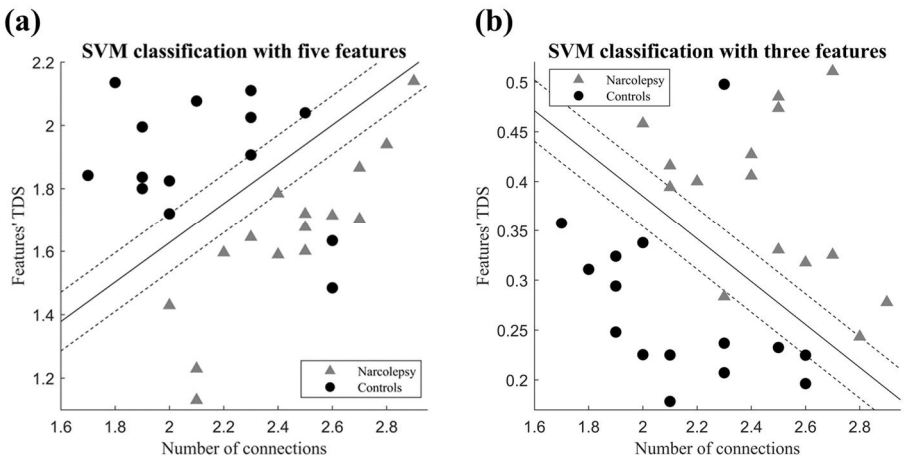

Figure 3-9. Distribution of participants with number of network connections and features' TDS during REM sleep. (a) contains five features including brain-brain connections and (b) contains three features not including brain-brain features. Solid lines represent the decision boundary to distinguish the two groups and dotted lines the parallel lines apart from decision boundary with margin distance. The number of connections multiplied by 0.1 to adjust scale of the figures.

3.2. Discussion

3.2.1. Differences between patients with narcolepsy and healthy controls

To analyze the differences in nocturnal sleep between patients with narcolepsy and controls, we used the TDS method to quantify and compare network connectivity values. Using data with minimized participant discrepancies, we found significant differences in nocturnal REM sleep, which is known to differ in patients with narcolepsy. TDS can be used to show relationships within a network how they persist in different physiological states. By using nine biosignals, we were able to determine the connectivity of the brain and peripheral areas during sleep.

The narcolepsy group showed a higher number of network connections during REM sleep than the control group (**Figure 3-1**). Compared to controls, patients with narcolepsy tended to maintain REM sleep with higher network connectivity among brain and body parts. Using TDS, we were able to measure how the participants maintained their states stably and how strong the connections were between organs. Separation of networks into three parts revealed significantly higher differences in the brain-periphery and periphery-periphery networks (**Figure 3-2**). θ-HR, HR-EYE, and CHIN-RESP significantly affected changes in network connection numbers (**Figure 3-3**). This means these connections had more power than others when interpreting the differences between the narcolepsy and control groups.

3.2.2. Analysis of nervous system with HRV

Connections that represent significant differences had physiological relationships that support the causality of network differences in both groups. As HR has a change in connections with θ and EYE in common, we also analyzed HRV. Before we accessed interactional relations with the heart to the brain and eyes, we conducted HRV analysis in the time and frequency domains to identify how cardiac information affects network connectivity independently within the autonomic nervous system. From the ECG data, we extracted every heartbeat during whole sleep using a self-developed automatic peak detection algorithm. The heartbeat was calculated using the time interval between the R-peaks of consecutive beats (RRI). This cardiac information was used to calculate parameters from the time and frequency domains. From the time domain, we obtained four parameters: HR, the standard deviation of RR intervals (SDNN), the root mean square of successive RR interval differences (RMSSD), and the percentage of successive RRI differing by more than 50 ms (pNN50) using the following equations:

$$\text{SDNN} = \sqrt{\sum_{i=1}^{N} \frac{RRI_i - \overline{RRI}}{N}} \quad \textbf{(3-1)}$$

$$\text{RMSSD} = \sqrt{\frac{\sum_{i=1}^{N-1} (RRI_{i+1} - RRI_i)^2}{N-1}} \quad \textbf{(3-2)}$$

$$\text{pNN50} = \frac{\sum_{i=1}^{N} \{|RRI_{i+1} - RRI_i| > 50\ ms\}}{N} \times 100 \quad \textbf{(3-3)}$$

where, RRI = RR interval, N = number of RRI

In the frequency domain, by applying fast Fourier transform (FFT) to the RRI, obtained the spectral power of the cardiac information. The spectral powers at the frequency ranges 0.04~0.15 Hz (LF; low-frequency) and 0.15~0.4 Hz (HF; high-frequency) were computed and normalized by dividing by the sum of the LF and HF. Additionally, the ratio of the normalized LF power to the normalized HF power (LF/HF) was extracted to examine the sympathovagal balance in the autonomic nervous system. The whole these time and frequency domain parameters were calculated within the range of a 90-s (three epochs) window. This was labeled the first epoch sleep stage, because we also used a 90-s window to define "stable" in the TDS method **[38-39]**. The window was shifted by 30 s (1 epoch) to calculate the HRV of every epoch and to perform an analysis based on sleep stage.

As a result, only RMSSD of the time domain in the REM sleep stage showed a significant difference between groups (p = 0.0498) (**Table 3-5**). A smaller RMSSD in the narcolepsy group indicates parasympathetic activation of the nervous system, which could lead to a stabilized heartbeat. This means that a lowered variance of heartbeat could lead to smaller fluctuations and induce higher synchronization in a stable state with other features **[40-43]**. Other HRV parameters did not differ significantly, but they followed the trends found in narcolepsy and control groups in previous studies **[44-46]**.

Table 3-5. HRV of time domain from participants with narcolepsy and controls

	Narcolepsy (N=15)				Controls (N=15)			
Time Domain	**HR**	**RMSSD**	**SDNN**	**pNN50**	**HR**	**RMSSD**	**SDNN**	**pNN50**
WAKE	71.84 ±9.61	40.47 ±17.89	83.03 ±26.59	9.28 ±7.08	66.98 ±4.91	54.81 ±28.99	87.13 ±30.16	12.77 ±9.02
REM	68.07 ±8.09	**38.9*** **±15.81**	62.54 ±16.26	8.94 ±6.38	64.43 ±6.72	56.53 ±36.41	75.99 ±42.07	11.24 ±9.19
Light Sleep	66.01 ±8.44	44.29 ±23.8	55.96 ±19.95	10.83 ±8.33	61.02 ±5.88	61.46 ±32.24	68.85 ±30.09	16.04 ±10.95
Deep Sleep	66.4 ±9.38	46.25 ±34.25	48.32 ±26.13	10.75 ±11.15	63.92 ±7.3	49.67 ±29.05	48.17 ±27.88	12.97 ±12.38

HR, Heart rate; RMSSD, Root mean square of the successive RR interval differences; SDNN, Standard deviation of the RR intervals; pNN50, Percentage of successive RR intervals that differ by more than 50 ms.

* $p < 0.05$ vs controls

3.2.3. Causalities in network connections

After HRV analysis, we used cardiac information to analyze the physiological relationships between connections. First in θ-HR connection, in REM sleep, it has been reported that the theta power of EEG is higher compared to other stages, and the theta rhythm is highly interrelated with heart rate with a positive correlation **[47-48]**. In addition, as narcolepsy showed a burst of theta waves in REM sleep compared to controls **[15,49-50]**, a burst of theta waves and heart rate could result in differences in connectivity between patients with narcolepsy and controls with higher correlation coefficients. We found a significantly higher brainwave power of theta in REM sleep than in other stages ($p < 0.05$) in both groups. In addition, the theta wave power in patients with narcolepsy was significantly higher during REM sleep than in controls ($p = 0.019$). Regarding the HR-EYE connection, heart rate and eye movement are also closely related. It is known that a rise in heart rate induces a decline in ocular pulse amplitude (OPA), which corresponds to eye movement **[51]**. Patients with narcolepsy in our study tended to have a higher heart rate than controls ($p = 0.102$), which could also cause differences in network connectivity related to the heart and eye. We also compared the correlation between two signals by extracting the results of time delay-based correlation coefficient values from cross-correlation [**37**]. The θ-HR connection showed a stronger positive correlation coefficient in patients with narcolepsy when compared to controls (**Figure 3-8-a,** Narcolepsy: 0.382 ± 0.118, Controls: 0.317 ± 0.122, $p = 0.05$), and patients with narcolepsy also showed stronger negative correlation coefficient values in the HR-EYE connection when compared to controls (**Figure 3-8-b,** Narcolepsy: -0.459 ± 0.126, Controls: -0.353 ± 0.101, $p = 0.023$). This might be interpreted to mean that different characteristics of the

physiological relationships between patients with narcolepsy and controls in REM sleep could lead to higher connectivity in the narcolepsy group. In addition, we speculate that θ-HR and HR-EYE connections in patients with narcolepsy may have stronger connectivity due to the effect of smaller RMSSD, which indicates smaller fluctuations of heart rate and, in turn, more synchronized brain and peripheral interactions compared to controls.

Additionally, in the CHIN-RESP connection, facial movement and breathing have meaningful causality, as the movement of facial muscles can disturb the airflow to the nasal or oral cavities **[52-53]**. In addition, the submentalis muscle (CHIN in our study) is known to have different REM sleep without atonia (RSWA) in patients with narcolepsy when compared to controls **[16]**. We calculated the phasic RSWA based on the AASM criteria **[34]**. Each 30-s epoch was divided into mini-epochs of 3 s; we used phasic movement to obtain the proportion of mini-epochs, which have more than four times higher EMG amplitude than the baseline EMG signal of the submentalis muscle. As a result, patients with narcolepsy showed a significantly higher proportion of phasic movement in their facial muscles during REM sleep than controls (Narcolepsy: 10.95 % ± 6.94 %, Controls: 3.38 % ± 1.07 %, $p < 0.001$). We infer that the loss of atonia during REM sleep may induce frequent movement during sleep, which, in turn, causes disturbances in breathing. The results of the correlation coefficient from the cross-correlation of TDS also support this interpretation, with a stronger negative correlation coefficient in patients with narcolepsy than in controls (**Figure 3-8-c,** Narcolepsy: -0.509 ± 0.092, Controls: -0.421 ± 0.095, $p = 0.018$). Therefore, in narcolepsy, we conclude that facial movement changes airflow in breathing. Facial movement and airflow may interact more closely and

spontaneously with each other with higher correlation and temporal resolution, which may lead to stronger connectivity with stability compared to controls.

3.2.4. Effect of brain-brain connections

Even though we chose to interpret brain-periphery and periphery-periphery connections, connections with brain-brain α-δ, θ-δ, which could not affect the number of network connections, might have their own meaning in the two groups during REM sleep. Unlike brain-periphery and periphery-periphery connections' distribution in **Figure 3-8**, brain-brain connections showed distributions of maximum time delay index mostly within a second, meaning that they are closely related, within small temporal window. Brain-brain connections also showed correlations' directionalities, however, correlations in both α-δ, θ-δ connections were higher in the control group than in the narcolepsy group, unlike brain-periphery and periphery-periphery connections (α-δ, Narcolepsy: 0.702 ± 0.111, Controls: 0.823 ± 0.125, p = 0.014), (θ-δ, Narcolepsy: 0.796 ± 0.142, Controls: 0.891 ± 0.177, p = 0.025). Based on these results, SVM performance with brain-brain area (**Figure 3-9-a**) could produce remarkable results which suggest the possibility that brain-brain networks could also indicate meaningful differences between groups during REM sleep.

3.2.5. Network connectivity as a biomarker and prospective utility

These results show that components reported as biomarkers to distinguish narcolepsy are closely related to network connectivity. As patients with narcolepsy are considered to have different eye and muscle movements and cardiac information, θ-HR, HR-EYE, and CHIN-RESP could be meaningful connections to validate their connectivity using the TDS method **[5,16]**. Higher connectivity in narcolepsy-related features supports the notion that differences (movement, cardiac information) in physiological connections between patients with narcolepsy and controls could lead to REM sleep stability, sustaining that state with increased connections. This suggests strong network connectivity in body systems could lead patients with narcolepsy to fall into REM sleep more easily and sustain a more stable REM state than controls. These findings and the classification performance with SVM indicate that network connectivity with the TDS method could be used as a useful biomarker to identify systemic network differences between patients with narcolepsy type 2 and controls. In addition, network connectivity could be used to determine causal interactions and help us understand the relationships among the body's system using quantified metrics. We anticipate this study could be useful reference and help to inspire new attempt to find adequate biomarkers in future studies to distinguish narcolepsy type 2.

In future studies, by analyzing the variations among sleep stages in detail with the TDS method, we can further examine network connectivity according to sleep stage transition. It would be possible to determine how network connections in the body change not only during sleep stages, but also during other transitions. There are several studies that have shown the existence of time delay among biosignals during

sleep, for example, changes in autonomic nervous system or brain-heart interaction according to sleep stage transition with time delay **[54-56]**. Thus, further study could reveal TDS trends that could be used to estimate the entering and escaping phases of the REM sleep state. Furthermore, with established techniques, we could estimate transitional changes in other states so that network connectivity could be used as a biomarker for other states not just for narcolepsy or REM sleep stage distinction.

4

Limitations

Our study analyzed differences in nocturnal sleep network connectivity between narcolepsy and control groups. However, there were some limitations worthy of consideration. First, although we have suggested several possible assumptions to interpret the differences between the two groups, there are limitations to explaining overall network connectivity; we only focused on significantly notable features. There should be additional clinical support to strengthen our understanding of complex human network systems. Second, the number of participants included may be insufficient for universal application. Even though our sample size was sufficient for analyzing statistical differences **[33]**, larger study samples would support our results and add more reliability. In addition, if we could conduct our study with a large number of participants, we could increase the model's robustness to classify the narcolepsy and control groups. Third, to assess this method from a therapeutic aspect, we should analyze changes in network connectivity for patients with narcolepsy who are undergoing treatment. We should perform a follow-up study to

determine whether this indicator is meaningful both before and after treatment. Fourth, to strengthen our assertion that network connectivity could be a meaningful biomarker, future studies should also analyze patients with narcolepsy type 1. Finally, as narcolepsy is diagnosed by implementing daytime MSLT, this process should be applied in daytime sleep. In this study, we also analyzed the differences in daytime sleep. The appearance of REM during daytime sleep, which we sought to analyze, is frequent in patients with narcolepsy but not in controls. This induces an imbalance of data for comparison. To overcome this limitation, a larger sample is needed.

Further experiments with proper control are warranted to examine the characteristics of narcolepsy in detail and to better understand the mechanisms behind physiological connections in the body.

5

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국문초록

렘 수면 중 기면 환자의 인체 시스템 내 생리학적 네트워크 연결성 변화

연구 배경: 기면증은 병리학적 증상을 수반하는 수면 질환의 하나로, 야간에 충분한 수면을 취했음에도 주간의 과도한 졸림증, 무기력증의 증상을 나타낸다. 기면증은 두 가지의 유형이 있으며 이들은 탈력발작을 동반한 1유형 기면증과 탈력발작을 동반하지 않는 2유형 기면증으로 구별된다. 1유형 기면증의 진단 생체 지표로서 히포크레틴이라는 신경물질이 존재하지만, 그에 반하여 2유형 기면증은 적절한 생체 지표가 부재하여 2유형 기면증의 기면 증상 및 인과관계의 확인에 한계를 지니고 있다. 이에 기반하여, 본 연구는 2유형 기면증의 새로운 생체 지표의 탐색을 목표로 하며 이를 위해 인체의 시스템적 연결망의 분석을 진행하였다.

연구 방법: 본 연구는 30명의 참여자 (15명의 2유형 기면증 환자, 15명의 정상 대조군)를 대상으로, 시간 지연 안정성의 방법을 통해 시간적 정보에 기반하여 여러 생체신호들의 관계에 대한 분석을 진행하였다. 각 참여자의 야간 수면다원 검사로부터 얻은 9개 생체신호들 (뇌파, 심장, 호흡, 근육과 안구의 움직임으로 부터의 신호)의 연결성 네트워크에 대한 정량적 분석을 진행하였으며, 특히 수면 단계에 따른 네트워크 연결성의 차이가 두 그룹 사이에 어떠한

영향력을 미치며 이들이 기면증과 정상군을 구별하는 잠재적 생체 지표로서 사용될 수 있을지에 대한 분석에 중점을 두었다. 그룹 간의 차이에 대한 인과관계의 조사와 함께 생체 지표의 분류 성능의 확인을 위한 서포트 벡터 머신 기법을 적용한 조사도 함께 진행하였다.

연구 결과: 렘 수면에서, 기면 환자군은 정상 대조군에 비교하여 더 많은 네트워크의 연결을 보였다 (기면 환자 연결 수: 24.47 ± 2.87, 대조군 연결 수: 21.34 ± 3.49; p = 0.022). 이러한 차이는 여러 연결의 요소들 중 움직임과 관련된 기관과 심장 활동에서 유의미하게 나타난 것을 확인할 수 있었으며, 네트워크의 연결 개수와 유의미한 차이를 보이는 생체신호 요소의 정보를 이용한 서포트 벡터 머신 기반 분류 성능은 0.93의 민감도, 특이도, 정확도를 각각 나타내었다.

결 론: 본 연구는 시간 지연 안정성에 기반한 네트워크 연결성이 2유형 기면증을 대조군과 분류하는 데에 있어 유용한 생체지표로 이용될 수 있음을 보이며, 나아가 인체의 시스템적 네트워크에 대한 분석을 통해 차이에 대한 인과관계 분석 및 정량적 접근이 가능함을 보여준다.

주요어: 기면증, 2유형 기면증, 뇌, 연결성, 렘 수면, 시간 지연 안정성

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