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RESEARCH ARTICLE

Control of Homeostasis

Linking stages of nonrapid eye movement sleep to the spectral EEG markers of the drives for sleep and wake

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Abstract

The conventional staging classification reduces all patterns of sleep polysomnogram signals to a small number of yes-or-no variables labeled wake or a stage of sleep (e.g., W, N1, N2, N3, and R for wake, the first, second, and third stages of nonrapid eye movement sleep and rapid eye movement sleep, respectively). However, the neurobiological underpinnings of such stages remained to be elucidated. We tried to evaluate their link to scores on the first and second principal components of the EEG spectrum (1PCS and 2PCS), the markers of two major groups of promoters/inhibitors of sleep/wakefulness delineated as the drives for sleep and wake, respectively. On two occasions, polysomnographic records were obtained from 69 university students during 50-min afternoon naps and 30-s stage epochs were assigned to 1PCS and 2PCS. Results suggested two dimensionality of the structure of individual differences in amounts of stages. Amount of N1 loaded exclusively on one of two dimensions associated with 1PCS, amounts of W and N2 loaded exclusively on another dimension associated with 2PCS, and amount of N3 was equally loaded on both dimensions. Scores demonstrated stability within each stage, but a drastic change in just one of two scores occurred during transitions from one stage to another on the way from wakefulness to deeper sleep (e.g., 2PCS changed from >0 to <0 during transition W→N1, 1PCS changed from <0 to >0 during transition N1→N2). Therefore, the transitions between stages observed during short naps might be linked to rapid changes in the reciprocal interactions between the promoters/inhibitors of sleep/wakefulness.

NEW & NOTEWORTHY In the present nap study, two dimensionality of the structure of individual differences in sleep stages was revealed. These results also suggested that individual variation in the sleep and wake drives associated with the first and second principal components of the EEG spectrum might underlie this structure. It seemed that each stage might be related to a certain, stage-specific combination of wake-sleep promoting/inhibiting influences associated with these drives for sleep and wake.

EEG spectrum; factor analysis; principal component analysis; sleep-wake regulation; ~~slow-wave sleep;~~ sleep staging

INTRODUCTION

The scientific study of human sleep has started with the discovery that, instead of being a homogeneous state of the brain, sleep proceeds through a series of distinct stages, and each stage has its own distinct brain wave pattern (1). Since the 1960s, the method of dividing sleep into such stages has become the essence of the generally accepted classification of substates of sleep. After introducing the conventional methodology of the standard sleep scoring rules (2), the attempts to revise these rules were rare. In particular, several different approaches to the description of sleep process were proposed as alternatives to discrete sleep stages after the introduction of computers into the processes of storage and analysis of electroencephalographic (EEG) records, e.g., Refs. 3–7 (for reviews, see Refs. 8, 9). Despite such developments, the originally proposed criteria for distinction between sleep stages in the EEG recorded on paper had remained almost

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unchanged in the past 50 years. For instance, the vast majority of these criteria have been included in the most recent and generally accepted classification of stages (10, 11).

As a rule, human sleep begins in stage 1 (N1) of nonrapid eye movement (NREM) sleep that is a transient state between wake (W) and sleep states. Sleep further progresses into stage 2 (N2), and then it progresses into stage 3 (N3), the deepest stage of sleep. The episodes of NREM sleep are usually preceding the first occurrence of sleep with rapid eye movements (REMs). Thus, the staging classification reduces all the patterns of sleep polysomnogram signals to a small number of yes-or-no variables labeled wake or a stage of sleep (e.g., W, N1, N2, N3, and R for wake, the first, second, and third sleep stages of NREM sleep, and REM sleep, respectively). However, this methodology ignores the question of the neurobiological underpinnings and validity of the subdivision into stages. Therefore, establishing a link of the formal staging approach to a neurophysiologically meaningful classification of sleep substates remains one of the unsolved scientific issues. Such questions remain to be open as why can we clearly differentiate one stage-specific pattern of brain waves from another, and what are the neurobiological underpinnings of such subdivision into stages? Previously, amount of only one of sleep stages, slow-wave sleep [stage 3 sleep or N3 in Iber et al. (10)], was directly linked to a process of regulating the sleep-wake cycle. The stage of deepest of sleep is considered a marker of homeostatic sleep pressure, and such EEG indicator of this stage as slow-wave activity (SWA) was used for quantitative description of the process of dissipation of this pressure governed by the drive for sleep (12, 13).

In some of the conceptualizations of the sleep-wake regulating processes, the two drives, the drive for sleep or the homeostatic process and the drive for wake or the circadian process, are interacting one with another to produce the circadian sleep-wake cycle (14, 15). This conceptualization of the sleep-wake regulating processes appears to be in line with the idea that complex neurobiological mechanisms of sleep and wakefulness can be, ultimately, delineated as oscillations between the processes promoting arousal and inhibiting sleep and the processes promoting sleep and inhibiting arousal (16–18). Moreover, the power of slow waves seems not to reflect the effect of the sleep drive alone, but it might represent the effect of reciprocal interactions between the drives for wake and sleep (19–21). The contributions of these sleep and wake drives to a current vigilance state can be separated by calculating scores on two largest (the 1st and 2nd) principal components of the EEG spectrum, the indicators of the strengths of the drives for sleep and wake, respectively (19–21).

It remains unexplored whether the reciprocal interactions between the drives for wake and sleep can explain other than N3 stages and the transitions between these stages. Moreover, it remains unexplored whether individual differences in amount of sleep stages can be related to individual variability in such markers of sleep and wake drives as scores on the first and second principal components of the EEG spectrum. Therefore, the purposes of the present nap study were *1*) to determine dimensionality of the structure of individual variation in amounts of sleep stages, *2*) to establish a link between this structure and the underlying individual differences in the EEG markers of the sleep and wake drives (i.e., scores on the first and second principal components of the EEG spectrum), and *3*) to describe the stages and transitions between them in terms of changes in strengths of the sleep and wake drives. The following hypotheses were examined:

1) the structure of individual differences in amounts of sleep stages can be reduced to two fully independent dimensions;
2) each of these two dimensions can be linked to the underlying individual variability in the two drives, for sleep and for wake;
3) each stage can be described in terms of a certain combination of the strengths of sleep and wake drives, and the transitions between these stages can reflect a rapid change in the strength of, at least, one the drives.

METHODS

All procedures performed in the studies were in accordance with the ethical standards of the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The protocols of the studies were approved by the Ethics Committees of the Institutes (No. 2 from June 3, 2019 and No. 5 from December 15, 2019). Each participant of the experimental study was informed in detail about all procedures and gave his/her written consent.

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Participants of Nap Study and Study Protocol

Unpaid volunteers of this study were 39 male and 30 female university students with mean ages of 19.3 and 18.9 yr (SD of 1.3 and 1.0, respectively). These students were invited by their lecturers to participate in the nap study and each participant was included in the study after the structured interview. To be included, a participant has to deny history of mental or sleep disorder, poor physical condition and functioning, current mild colds, missing classes due to any sickness in the previous 2 wk, involvement in shift or night work, crossing several time zones during the previous month, irregular sleep-wake schedule (i.e., more than 1-h difference in weekday bedtimes), frequent sleep deprivation (i.e., at least, 1 day of sleep deprivation in the previous week), and ages either younger than 18 or older than 22 yr. None of female participants was pregnant or on breastfeeding.

A 50-min polysomnographic record of napping attempt was obtained twice with an interval between such records varying from 3 days to 1 mo. Each visit to sleep laboratory lasted for less than 1 h and a half and approximate time of arrival in laboratory was 1:00 PM. The visit was preceded and followed by attending classes within the same building.

Polysomnographic Recordings and Sleep Scoring

A participant went to bed under exposure to dim light (~10 lx), and the electrodes were applied for polysomnographic recordings on a 16-channel wireless system ("Neuropolygraph 24," Neurotech, Taganrog, Russia). Standard monitoring montage included 13 EEG channels, one chin electromyogram channel, and two electrooculogram channels. All electrodes were placed in accord with the International 10–20 system of electrode placement.

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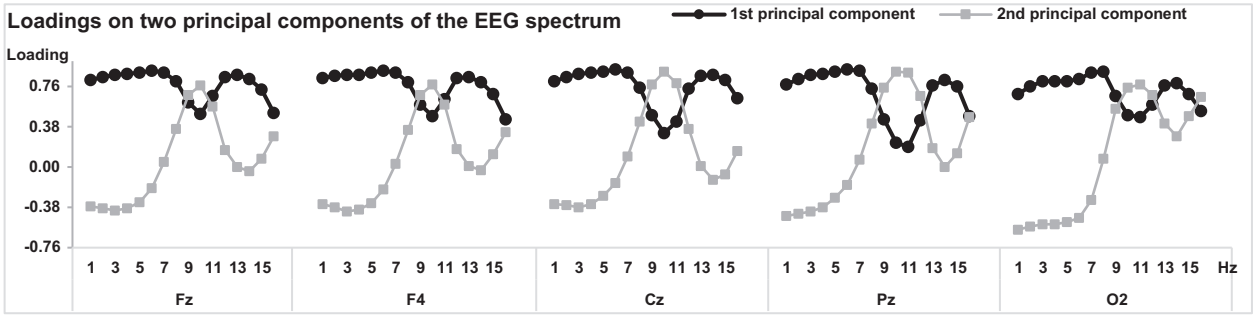

Figure 1. Loadings of 16 powers (1–16 Hz) from five derivations on principal components. The analysis was performed on 30-s spectra calculated for 30-s epochs of two naps for each of five derivations, Fz, F4, Cz, Pz, and O2 (n = 69 individuals × 2 naps × 100 epochs per nap). First and second principal components: two largest principal components yielded by principal component analysis of 16 ln-transformed single-Hz power densities. See variation explained by these two principal components in Supplemental Table S1.

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During applying the electrodes, a participant was instructed to try to relax and to nap for the following 50 min after light off.

The recorded signals were conditioned by the high-pass, low-pass, and notch filters (frequencies of 0.5, 35, and 50, respectively), sampled on a hard disk with a frequency of 500 Hz, and stored. Visual scoring on 30-s epochs of each 50-min record was performed in accord with the conventional scoring procedure (10). Two experienced scorers scored each record independently with the initial disagreement varying, depending upon a stage, from 15% (N1) to 5% (N3). They finally reexamined together all intervals with discrepant scores to produce consensus scores. The 30-s epochs were classified into five stages: wake stage (W), REM (rapid eye movement) sleep (R), and three stages of NREM (NREM) sleep (stage 1 sleep or N1, stage 2 sleep or N2, and slow-wave sleep or N3). Because the epochs with R were very rare, this stage was not included in the analysis as a separate sleep stage.

Analysis of the EEG Signals

The EEG signals from electrodes placed at five derivations (Fz, F4, Cz, Pz, and O2 referenced to the ear mastoid sites, M1/M2) were used for calculating the EEG power spectra densities. Before the calculation, the records of the signals from these derivations were visually inspected on 1-s epochs to remove all epochs containing artifacts from further analysis. Power spectra densities for the artifact-free epochs were computed using the FFTW (Fastest Fourier Transform in the West) package (22) (see www.fftw.org for more detail). Hamming window taper was used on 1-s epochs to calculate absolute spectral power densities (μV^2) for each of the first 16 single-Hz frequency bandwidth, i.e., 0.50–1.49 Hz (1 Hz), 1.50–2.49 Hz (2 Hz), 2.50–3.49 Hz (3 Hz), . . ., 15.50–16.49 Hz (16 Hz). These sets each of which consisted of 16 single-Hz power densities (1–16 Hz) were averaged over 30-s intervals of EEG records (a number of averaged 1-s epochs varied from 5 to 30).

For statistical analyses, the individual powers were ln-transformed and further averaged, e.g., over derivations, over five 10-min intervals of the records, over each of two napping attempts, within four 4-Hz frequency ranges of δ (1–4 Hz), θ (5–8 Hz), α (9–12 Hz), and σ (13–16 Hz) powers, etc.

Statistical Analysis

For statistical analysis, the $SPSS_{23.0}$ statistical software package (IBM, Armonk, NY) was used. The sets of 16 ln-transformed single-Hz power densities (1–16 Hz) from each of five derivations were subjected to principal component analysis (Fig. 1, Table 1, and Supplemental Table S1; all supplemental material is available at https://doi.org/10.6084/m9.figshare.17041247). Scores on the first and second principal components of variation in the EEG power spectra were calculated and subjected to further analysis (Tables 2 and 3, Supplemental Tables S2 and S3, Figs. 2 and 3, and Supplemental Figs. S1 and S2). For sleep and spectral EEG indexes obtained in two naps, Cronbach's α was computed as one of two reliability (internal consistency) measures (Table 2, *left*). The Pearson coefficient of correlation was applied for estimation of another (test-retest) reliability measure (Table 2 and Supplemental Table S2) and for checking

Table 1. *Loadings on two principal components*

	1st Nap		2nd Nap	
Hz	1st	2nd	1st	2nd
1	***0.87***	**−0.40**	***0.80***	**−0.50**
2	***0.89***	−0.39	***0.83***	**−0.48**
3	***0.91***	−0.37	***0.86***	**−0.47**
4	***0.92***	−0.34	***0.89***	**−0.43**
5	***0.93***	−0.29	***0.92***	−0.36
6	***0.95***	−0.22	***0.95***	−0.23
7	***0.96***	−0.08	***0.95***	0.02
8	***0.91***	0.19	***0.82***	0.38
9	***0.72***	**0.55**	**0.55**	***0.74***
10	**0.58**	**0.74**	0.38	***0.86***
11	***0.64***	**0.71**	**0.49**	***0.78***
12	***0.84***	0.34	***0.80***	**0.43**
13	***0.90***	0.04	***0.90***	0.06
14	***0.88***	−0.07	***0.87***	−0.07
15	***0.83***	0.03	***0.81***	0.12
16	***0.70***	0.23	**0.58**	**0.48**
E	11.5	2.3	10.0	3.5
%	71.6	14.3	62.6	22.1

Loadings on the two largest (1st and 2nd) principal components yielded by principal component analysis of 16 ln-transformed single-Hz power densities. The analysis was performed on the sets of individual spectra for naps first and second obtained by averaging over all 100 30-s spectra of an individual (n = 69). E and %: eigenvalue and percentage of total variance explained. Loadings higher than 0.4 or lower than −0.4 are printed in bold, and those higher than 0.6 or lower than −0.6 are printed in bold italic.

Table 2. *Two reliability measures and correlations with principal component scores*

Spectral EEG Index or Amount of stage	Reliability		Correlation with Principal Component	
	α	Test-Retest	Score: 1st	2nd
1st score	***0.843***	***0.730******	***1.000***	0.156
2nd score	***0.806***	***0.684******	0.156	***1.000***
δ power	***0.734***	**0.584*****	***0.874******	−0.285*
θ power	***0.837***	***0.719******	***0.970******	0.115
α power	***0.888***	***0.799******	***0.682******	***0.779******
σ power	***0.890***	***0.807******	***0.858******	0.246*
Amount of W	***0.672***	**0.527*****	−0.355**	***0.663******
Amount of N1	0.384	0.249*	**−0.507*****	−0.155
Amount of N2	**0.433**	0.277*	0.348**	**−0.444*****
Amount of N3	**0.438**	0.286*	**0.559*****	**−0.431*****

First and second score: scores on the first and second principal components yielded by principal component analysis of 16 ln-transformed single-Hz power densities; each spectral EEG index was obtained by averaging over five derivations. Reliability: α and test-retest: Cronbach's α (internal consistency measure) and Pearson correlation coefficient. Level of significance for Pearson correlation coefficient: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$; correlation coefficients higher than 0.4 are printed in bold, and those higher than 0.6 are printed in bold italic.

significance of correlation of each of two principal component scores with amounts of sleep stages (Table 2, *right*). Amounts of four stages were subjected to factor analysis with varimax rotation to determine the number of factors with eigenvalue higher than 1, rotate these factors, and estimate loadings of amounts of stages on each of the rotated factors (Table 3). The procedure was repeated with adding either spectral powers in four frequency regions (δ, θ, α, and α) or two scores (the first and second) on principal components of the EEG spectrum (Table 3). Moreover, the study participants were subdivided into two subsamples in accord with either higher or lower amount of each of four stages (Fig. 2 and Supplemental Fig. S1). Significance of main effect of independent factor "Subsample" (either higher or lower amount of a stage) was checked by applying two-way MANOVA with "~~Gender~~" as another (second) independent factor (Supplemental Table S3).

AQ: 9

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In addition, the time courses of the first and second principal component scores were calculated for four stages (Fig. 3*A*). The time courses were also calculated on the intervals preceding and following the onset of each of four stages (Fig. 3*B*). Paired *t* test was applied to compare scores on these preceding and following intervals. Level of significance of each statistical result was corrected to account for the number of compared pairs (see notes to Table 4). T4

RESULTS

Calculation of Scores on the First and Second Principal Components of the EEG Spectrum

Depending upon derivation (Fz, F4, Cz, Pz, and O2), the two largest principal components of power spectra (the first and second) are explained from 76.3% to 82.3% of total variance in 16 ln-transformed single-Hz power densities (Table 1 and Supplemental Table S1). Eigenvalues of the third component were either equal or somewhat lower than 1, and the eigenvalues of any of remaining components were always <1. In the results of analysis performed after averaging 30-s spectra over 100 epochs of the nap interval and five derivations ($n = 69$), the pattern of loadings on the first and second principal components (Fig. 1 and Supplemental Table S1) remained unchanged (Table 1). This pattern of loading suggested that only α frequencies positively loaded on both components. δ Frequencies positively loaded on the first principal component and negatively loaded on the second principal component, and σ and θ frequencies positively loaded on the first principal component (Fig. 1 and Table 1). For further analyses (Figs. 2 and 3, and Tables 2, 3, and −4, Supplemental Tables S2 and S3), scores on the first and second principal components of variation in 30-s power spectra were averaged over derivations.

Test-Retest Reliability of Sleep Stages and Spectral EEG Indexes

Principal component scores calculated for two napping attempts significantly correlated one to another. Table 2

Table 3. *Loadings on two varimax rotated factors*

Amount of Stage or Spectral EEG Index	4 Sleep Stages		With 4 Powers		With 2 Scores	
	1st F	2nd F	1st F	2nd F	1st F	2nd F
Score 1st					−0.159	***0.857***
Amount of N1	0.118	***0.939***	***−0.726***	−0.033	−0.162	***−0.856***
Amount of N3	**0.518**	***−0.746***	**0.419**	***0.678***	**−0.538**	***0.682***
Amount of N2	***0.883***	−0.043	0.011	***0.823***	***−0.781***	0.206
Amount of W	***−0.984***	0.072	0.069	***−0.981***	***0.956***	−0.173
Score 2nd					***0.839***	0.198
δ power			***0.653***	***0.687***		
θ power			***0.845***	**0.432**		
α power			***0.859***	−0.275		
σ power			***0.759***	0.257		
Eigenvalue: initial (IE) and after rotation (AE) and %						
IE	2.221	1.254	4.062	2.002	2.893	1.721
AE	2.030	1.445	3.163	2.901	2.568	2.045
%	50.76	36.12	39.54	36.26	42.81	34.09

Loading on varimax rotated factors: loadings on two largest varimax rotated factors, 1st F and 2nd F, with initial eigenvalue > 1; %: percentage of total variance explained by each of two factors with initial eigenvalue (IE) > 1; with 2 scores: scores on the first and second principal components yielded by principal component analysis of 16 ln-transformed single-Hz power densities. Loadings higher than 0.4 or lower than −0.4 are printed in bold, and those higher than 0.6 or lower than −0.6 are printed in bold italic.

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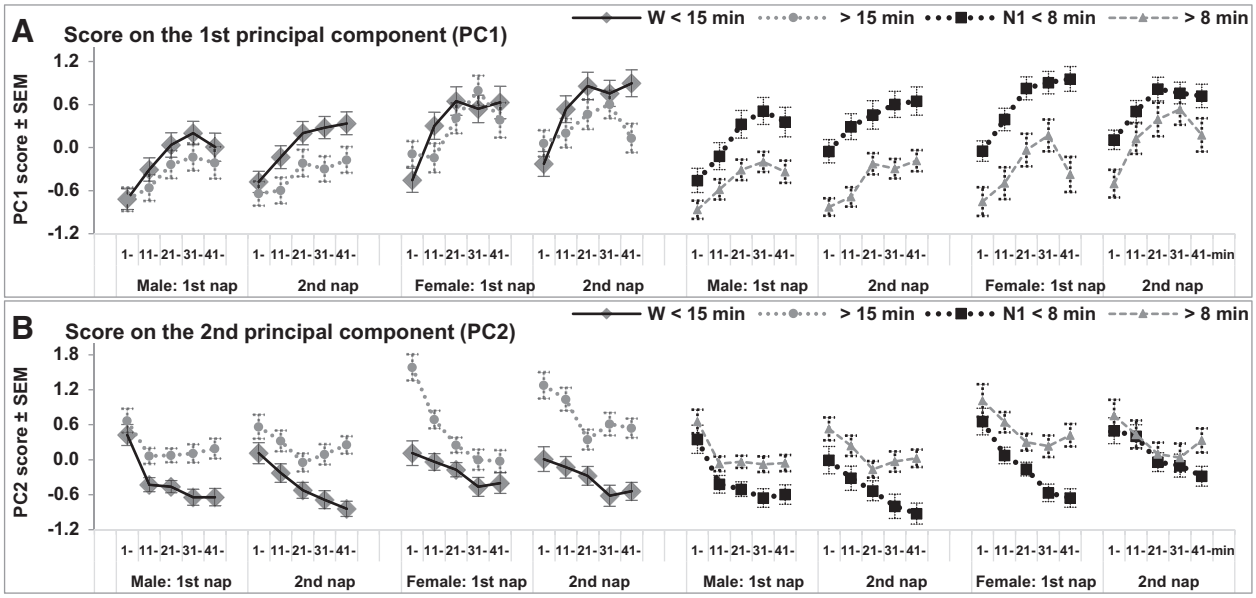

Figure 2. Time courses of principal component scores in subsamples differed in two stages. *A* and *B*: scores on the first and second principal components, respectively, on 10-min intervals of 50-min record (1-, 11-, 21-, 31-, and 41-) for two subsamples. The division into subsamples was performed in accord with a criterion, either < or > 15 min for W (*left*) and either < or > 8 min for N1 (*right*). Male and female: subsets of data for male and female students; first and second nap: the first and second napping attempt. See Supplemental Table S3 for statistical significance of results on the differences between subsamples in scores.

provides the comparison of strengths of these correlations with the strengths of correlations calculated for traditional EEG indexes and amounts of four stages. The correlations between scores on principal components were slightly weaker than those for spectral powers in faster frequency bands (α and σ) but slightly stronger than the correlations in the slowest (δ) frequency band (Table 2). The results shown in Table 2 also suggested significant albeit weaker correlations between each pair of the amounts of stages in two napping attempts. Moreover, results included in Supplemental Table S2 suggested stability of the patterns and strengths of correlations calculated for each of five 10-min intervals of 50-min napping attempt. In contrast, the correlations obtained for amounts of four stages on

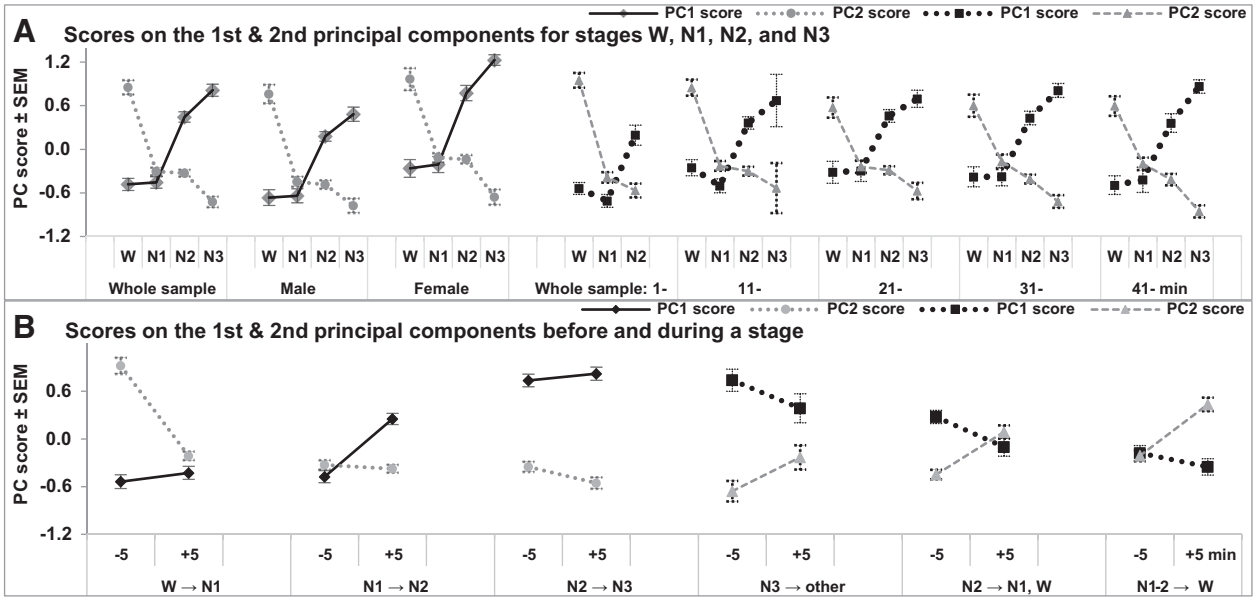

Figure 3. Scores on principal components for stages. *A*: scores on the first and second principal components calculated for four separate stages. Whole sample: scores averaged over the whole nap interval (50 min); 1-, 11-, 21-, 31-, and 41-: scores averaged on five 10-min intervals of napping attempt; *B*: change in scores on the first and second principal components of the EEG spectrum during transitions from one stage to another; −5 and +5: Up to 5 min before and up to 5 min following the transition to a given stage, respectively. See Table 4 for the results of *t* tests checking significance of change in scores.

Table 4. *Paired t test for differences between principal component scores on 5-min intervals*

Score on the		1st Principal Component				2nd Principal Component			
Min	Stage(s)	Difference	*t*	df	*P*	Difference	*t*	df	*P*
Sleep deepening from W to N1 to N2, and to N3, and through each of the stages									
−5▸+5	W▸N1	−0.110	−2.26	67	0.027	***1.134***	***14.66***	***67***	***<0.001***
+5▸−5	→N1→	0.005	0.11	65	0.912	**0.094**	**3.25**	**65**	**0.002**
−5▸+5	N1▸N2	***−0.728***	***−16.3***	***65***	***<0.001***	0.049	1.20	65	0.231
+5▸−5	→N2→	***−0.404***	***−9.31***	***44***	***<0.001***	−0.018	−0.38	44	0.700
−5▸+5	N2▸N3	**−0.086**	**−3.52**	**44**	**0.001**	**0.203**	***7.25***	***44***	***<0.001***
+5▸−5	→N3→	−0.014	−0.69	20	0.497	0.116	2.43	20	0.025
Sleep lightening from N3 to N2 to N1, and to W									
−5▸+5	N3▸Other	**0.353**	**3.62**	**20**	**0.002**	***-0.425***	***−4.24***	***20***	***<0.001***
−5▸+5	N2▸N1, W	***0.379***	***5.13***	***49***	***<0.001***	***-0.536***	***−7.80***	***49***	***<0.001***
−5▸+5	N1, N2▸W	0.173	2.81	52	0.007	***-0.647***	***−7.97***	***52***	***<0.001***
Remaining transitions									
+5▸−5	Other▸N2	−0.041	−0.27	18	0.784	0.195	1.60	18	0.126
+5▸−5	W, N1▸N1, N2	0.136	1.69	43	0.097	***0.427***	***4.88***	***43***	***<0.001***

Paired student's *t* test for testing significance of changes in principal component scores in a sequence of transitions between four stages illustrated in Fig. 3B. Difference: averaged difference between mean amount of stage(s) calculated on 5-min intervals, either up to 5 min of the EEG record preceding to a stage (−5) or during the following up to 5 min of this stage (+5), positive difference indicates a decrease of score, whereas negative difference indicates an increase of score; *t*, df, and *P*: *t* statistic, degree of freedom, and level of significance; changes in a score that are statistically significant with $P < 0.001$ and $P < 0.0045$ are printed in bold and bold italic (the results remained significant after correction for 11 comparisons, $P = 0.05/11 = 0.0045$), respectively.

separate 10-min intervals often did not reach a statistically significant level (Supplemental Table S2).

Therefore, the results on reliability of the EEG indexes and amounts of stages (Table 2 and Supplemental Table S2) suggested that it was lower for any of four stages and much higher for any of the spectral EEG indexes, including the spectral EEG markers of the sleep and wake drives (e.g., scores on 2 principal components). Given Cicchetti's (23) suggestion to define 0.40–0.59 as fair test-retest reliability, 0.60–0.74 as good, and above 0.75 as excellent test-retest reliability, reliability can be regarded as being fair for amounts of stages, but good for the spectral EEG markers of sleep and wake drives.

Dimensions of Individual Variation in Amounts of Sleep Stages

The results of factor analysis yielded two dimensionality of individual variation in amounts of four stages (Table 3). Not more than two orthogonal dimensions were necessary to account for the major portion of individual variation in amounts of these stages (see bottom lines of Table 3). The results also indicated that, although amount of N1 loaded exclusively on one dimension~~, amounts of~~ W and N2 loaded exclusively on another dimension, and amount of N3 equally loaded on both dimensions (Table 3).

Moreover, the results shown in the right pair of columns of Table 3 with the results of the joint analysis of the principal component scores and amounts of four stages suggested a differential relationship of these scores with two dimensions. Moreover, the same three major patterns of relationship with amounts of stages were obtained and these patterns yielded by such a joint analysis were identical to the three patterns revealed by the sole analysis of amounts of four stages (Table 3). Finally, these patterns were the same as the three patterns yielded by the correlation analysis (Table 2, *right*). Namely, amount of N1 showed the associations with the first score and with one dimension (Table 2, *right*, and Table 3). Amount of W was associated with the second score and with another dimension (~~Table~~, *right*, and Table 3). Amount of N2 showed approximately the same strength relationship as did amount of W, but the direction of association was the opposite (i.e., if amount of W loaded or correlated positively, amount of N2 loaded or correlated negatively). As for amount of N3, it was equally associated with both scores and with both dimensions (Table 2, *right*, and Table 3).

Therefore, the results of the factor and correlation analyses (Table 2, *right*, and Table 3) suggested two dimensionality of the structure of individual variation in amounts of four stages and revealed a close link of individual differences in amounts of these stages to individual variability in two principal component scores proposed to be the markers of wake and sleep drives underpinning these two independent dimensions of individual variation and sleep stages.

Comparison of Subsamples Differed in Amount of a Stage

Figure 2 and Supplemental Fig. S1 illustrate the results of MANOVA (Supplemental Table S3) providing further support to the results of factor and correlation analyses of individual variation in four stages and two principal component scores (Supplemental Table S2, *right*, and Table 3). They suggested that amounts of W and N2 were strongly determined by individual variation in the wake drive marker (2nd score), whereas the sleep drive marker (1st score) did not contribute to individual variation in this amount (Fig. 2 and Supplemental Fig. S1). In contrast, individual variation in N1 was strongly determined by individual variation in the sleep drive marker, whereas the wake drive marker did not contribute to individual variation in this amount (Fig. 2). The results on amount of N3 suggested that slow-wave sleep was the lowest in amount when a weak sleep drive was combined with a strong wake drive, i.e., the largest and the lowest amounts were found for the opposing one another combinations of the strengths of the drives (Supplemental Fig. S1).

Therefore, these results suggested that, only for amount of N3, the opposing one another influences of the sleep and wake drives was revealed by MANOVA. In contrast to the joint contribution to amount of N3, the drives differentially contributed to the amounts of three other stages. A weak wake drive alone determined a small amount of W and a large amount of N2, whereas a weak sleep drive alone determined a large amount of N1. In overall, the results of the MANOVA confirmed that the structure of individual differences in amounts of stages can be linked to the underlying individual variation in the markers of sleep and wake drives (Supplemental Table S3, Fig. 2, and Supplemental Fig. S1).

Comparison of Principal Component Scores within and between Stages

Figure 3 clarifies the differential relationship of amounts of stages with the spectral EEG markers of sleep and wake drives. Low score on the first principal component in both W and N1 might be interpreted as indicating that the drive for sleep remained inhibited (switched-off) not only during wakefulness but also during transition from wakefulness to sleep (W→N1). Although the first score remained low (the sleep drive is switched-off), significant decline of the second score occurred during such a transition (Table 4 and Fig. 3) indicating that the duration of W was exclusively determined by the change in wake drive state (from switched-on to switched-off). The weaker was the drive, the shorter was W, and this transition occurred earlier. In contrast, the second score that rapidly changed during the transition from W to N1, remained unchanged during the following transition, from N1 to N2 (the underlying drive for wake remained in switched-off state). Instead, the first score rapidly increased during N1→N2 transition (Table 4 and Fig. 3). This might be interpreted as indicating a rapid change in the state of sleep drive from switched-off to switched-on. The buildup of this first score suggested that the duration of N1 was exclusively determined by the sleep drive. The weaker was the drive, the longer was N1, and this transition occurred later. Furthermore, the duration of N2 was mostly determined by the wake drive. The weaker was the drive, the longer was N2, and the next transition (from N2 to N3) occurred later (Table 4 and Fig. 3). N2→N3 transition was associated with a smaller shift in both scores that usually was not the change in sign of a score. This might be interpreted as indicating the further manifestation of the influence of wake drive indicated in further decline of the second score (from <0 to $<<0$), and the further manifestation of the influence of wake drive indicated further increase of the first score (from >0 to $>>0$).

The results on time courses of principal component scores (Table 4 and Fig. 2 and Supplemental Fig. S1) suggested that each stage seemed to have its unique and rather stable EEG spectrum reflecting a stage-specific combination of the strengths of drives for sleep and wake. Individual variation in amount of one stage was fully independent from individual variation in amount of another stage because the durations of these stages were determined by two separate drives. For example, amount of W was determined by the wake drive represented by the second principal component score, but not by the sleep drive represented by the first principal component score; amount of the following stage N1 was determined by the sleep drive but not by the wake drive; and amount of N3 seemed to be the only stage which amount was approximately equally dependent upon any of the drives.

Stages, Transitions between Them, and Promoters/Inhibitors of Sleep

It seemed that the drives for sleep and wake that are associated with the reciprocal promoters/inhibitors of sleep and wakefulness tended to remain in the same state (either on or off) throughout the entire stage. Moreover, the drives usually were not simultaneously involved in the initiating transition from one stage to another by means of changing a state of a drive either from off to on or from on to off on the way from relaxed wakefulness through light sleep to deeper sleep and to the deepest sleep (from W to N1, from N1 to N2, and from N2 to N3, respectively). First, the change in state of the wake drive from "on" to "off" occurred during W→N1 transition. Second, the change in state of the sleep drive from "off" to "on" occurred during N1→N2. Third, because the states of the wake and sleep drives during N1 and N2 might remain unstable, their stabilization occurred during N2→N3 transition (Supplemental Fig. S2).

In overall, the results reported in Figs. 2 and 3, Supplemental Figs. S1 and S2, and Tables 2, 3, and −4 suggested that individual differences in the duration and proportion of sleep-wake stages might reflect individual differences in the strengths of the sleep and wake drives measured by scores on the first and second principal components of the EEG spectrum, respectively. Positive score obtained by averaging over study participants might be interpreted as indicating a switched-on state of a drive, whereas negative score be interpreted as indicating a switched-off state of a drive. As individual differences in amounts of the stages might be linked to individual differences in scores of the first and second principal components of the EEG spectrum that are proposed to be the markers of the underlying drives for sleep and wake, respectively, the durations and proportions of particular stages on the 50-min interval of polysomnographic nap recording might indicate the strengths of the drives (Supplemental Fig. S2). The chain of transitions from wake state to deep sleep observed within the 50-min interval of polysomnographic nap record consists of a sequence of abrupt changes in states of the drives linked to the transitions from one stage to another (Fig. 3).

DISCUSSION

Scientific study of human sleep has been initiated by the discovery that sleep is not a homogeneous state of the brain but progresses through a series of stages in which different brain wave patterns are displayed [e.g., Loomis et al. (1)]. This and several following discoveries of associations between the pattern of brain waves and sleep substates led to the development of methodology allowing an economical quantitative description of sleep by subdividing a sleep record into intervals each of which is allocated to one of a few all-or-nothing variables called "sleep stages" (2, 10). However, it remains to be explored whether these relatively stable stages and the transitions between them can be linked to the states of the sleep-wake regulating

mechanisms and the changes in these states, respectively. Here, we explored the structure of individual variation in four stages in relation to individual differences in the first and second principal component scores that were proposed to be the spectral EEG markers of strengths of sleep and wake drives, respectively. This structure was found to be two dimensional and each of two orthogonal dimensions demonstrated a link to one of two scores. Individual differences in amount of any of four stages were found to be associated with individual variation in a score, with the first score representing strength of sleep drive (N1), with the second score representing strength of wake drive (W and N2), and with both scores (N3). It was found that scores on the first and second principal components of the EEG spectrum did not change much within a stage, whereas a drastic change in one of two scores was identified during a transition from one stage to another (i.e., during the transitions W→N1→N2, the changes in a score from <0 to >0 or from >0 to <0 were observed). Therefore, we interpreted the transitions from relaxed wakefulness through lightened sleep to deepened sleep (W→N1→N2→N3) in terms of switch between the states of one or two drives. There is a possibility to distinguish between four not just two stages because the states of the drives during the transitions W→N1→N2 do not change simultaneously from on (or off) to off (or on). Instead, strength of only one of the underlying drives (e.g., either the wake drive or the sleep drive) determined amount of a stage (e.g., either W or N1 or N2). Consequently, there exists a possibility to establish a link between amount of stages and a stage-specific combination of the states of drives for sleep and wake (i.e., the representatives of two major groups of promoters/inhibitors of sleep/wakefulness). Such results provided a possibility to describe the stages in terms of two major processes of regulation of the sleep-wake cycle, i.e., as a state or substate of sleep-wakefulness occurring due to the reciprocal interactions between sleep- and wake-promoting processes that inhibit one another in the course of transitions between these states and substates. The capturing of this fundamental feature of sleep-wake regulating mechanisms by the Rechtschaffen and Kales (2) rules can, at least partly, explain their success in providing the continuity of the scientific and clinical description of the sleep process for a half of century.

In theoretical terms, the results pointed at the possible neurobiological underpinning of the division of the sleep-wake cycle into the stages. They provided the plausible answers to such questions as why it is possible to subdivide the sleep-wake cycle into stages in accord with their specific patterns of brain wave activity and how the difference between people in the duration and proportions of these stages can be interpreted. It seems that the subdivision into stages might be linked to the concept of complex neurobiological mechanisms of sleep and wakefulness delineated as oscillations between the processes promoting arousal and inhibiting sleep and the processes promoting sleep and inhibiting arousal (e.g., Refs. 16–18). In agreement with this concept and in the light of the concept of regulation of the sleep-wake cycle by the sleep and wake drives (14, 15, 19–21), the transitions between stages can be viewed as reflecting abrupt changes in the state of these drives. Moreover, the differences between people in the duration of these stages can be linked to the differences in strengths of these two drives. As the duration of napping attempt in the present study was relatively short, the results did not allow the assessment of individual differences in R. Due to similarity of the EEG spectra for R and W, the explanation of this stage might require a proposition of an additional regulator for promoting this stage but inhibiting arousal despite the resemblance of principal component scores for wake state and REM sleep.

In practical terms, further studies might help to identify whether people with good and disturbed sleep are different in such EEG markers of sleep and wake drives as scores on the first and second principal components. If yes, such a difference in scores can provide a possibility to establish a link of sleep disturbances to the parameters of processes of sleep-wake regulation.

The literature on individual variation in duration and proportions of stages allows the conclusion that amounts of sleep stages might exhibit large individual differences constituting a trait (24). For instance, profound and systematic individual differences were reported for slow-wave sleep (N3). They were found to be stabile across multiple nights of sleep (24), nighttime naps (25), and daytime naps (26). Moreover, individual variation in spectral EEG markers of the underlying sleep-wake regulating processes seems to constitute a trait (27–30). For example, either good or excellent test-retest reliability between two consecutively recorded nights was reported for different indexes of slow-wave activity (31). Moreover, the conventional spectral EEG characteristics (powers in δ and other frequency ranges) were mentioned among the most heritable traits in humans, with the highest hereditability shown by spectral power in α frequency band (32–34). A significant correlation coefficient between amounts of N3 in the first and second napping attempt obtained in the present study was in agreement with previous findings on N3 (24–26). Moreover, the strengths of correlations between spectral EEG measures obtained in the first and second napping attempts were in agreement with those reported in the literature (e.g., Refs. 27–31). Besides, the present results allowed the conclusion that reliability estimates were lower for amounts of stages and higher for the spectral EEG indexes including scores on the first and second principal components of the EEG spectrum.

Conclusions

In the present nap study, two dimensionality of the structure of individual differences in sleep stages was revealed. These results also suggested that individual variation in the sleep and wake drives associated with the first and second principal components of the EEG spectrum might underlie this structure. It seemed that each stage might be related to a certain, stage-specific combination of wake-sleep promoting/inhibiting influences associated with these drives for sleep and wake. In particular, it was found that amount of a stage might be associated with the states of the underlying drives, i.e., with a state of either sleep drive (N1) or wake drive (W and N2) or both (N3). Therefore, scoring the EEG markers of these two drives might allow the distinguishing between states of each drive, e.g., a score might be either

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higher or lower than 0 thus reflecting either on or off state of a drive, respectively.

SUPPLEMENTAL MATERIAL

Supplemental Tables S1–S3 and Figures S1 and S2: https://doi.org/10.6084/m9.figshare.17041247.

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AQ: 15

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AQ: 16

AUTHOR CONTRIBUTIONS

V.B.D., O.N.T., D.S.S. and A.A.P. conceived and designed research; A.O.T., S.S.G., O.N.T., D.S.S., Z.B.B., and A.A.P. performed experiments; D.S.S. and A.A.P. analyzed data; V.B.D. and A.A.P. interpreted results of experiments; A.A.P. prepared figures; D.S.S. and A.A.P. drafted manuscript; A.A.P. edited and revised manuscript; V.B.D., A.O.T., D.S.S., S.S.G., O.N.T., D.S.S., Z.B.B., and A.A.P. approved final version of manuscript.

AQ: 17

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