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Principal Component Structuring of the Non-REM Sleep EEG Spectrum in Older Adults Yields Age-Related Changes in the Sleep and Wake Drives

Arcady A. Putilov[1]*, Mirjam Y. Münch[2, 3] and Christian Cajochen[2]

[1]*Research Institute for Molecular Biology and Biophysics, 2, Timakova Street, 630117 Novosibirsk, Russia;* [2]*Centre for Chronobiology, Psychiatric Hospital of the University of Basel, Wilhelm-Kleinstr. 27, CH-4012 Basel, Switzerland;* [3]*Current address: Solar Energy and Building Physics Laboratory, École polytechnique fédérale de Lausanne (EPFL), Station 18, CH-1015 Lausanne, Switzerland*

Abstract: Age-related disturbances of the sleep-wake cycle can reflect ontogenetic changes in regulatory mechanisms underlying normal and pathological aging, but the exact nature of these changes remains unclear. The present report is the first attempt to apply principal component analysis to the electroencephalographic (EEG) spectrum to examine of whether the observed age-related changes in the objective sleep measures can be linked to the opponent sleep-promoting and wake-promoting processes. The EEG indicators of these processes - scores on the 1st and 2nd principal components of the EEG spectrum, respectively - were compared in 15 older (57-74 years) and 16 younger (20-31 years) healthy volunteers. The scores were calculated for non-REM sleep episodes which occurred during ten 75-min naps scheduled every 150 min throughout a 40-h constant routine protocol. Both, a decrease of the 1st principal component score and an increase of the 2nd principal component score were found to contribute to such most obvious age-related modification of the sleep EEG spectrum as attenuation of EEG slow-wave activity in older people. Therefore, we concluded that the normal aging process can reflect both a weakening of the sleep-promoting process and a strengthening of the wake-promoting process, respectively. Such bidirectional changes in chronoregulatory processes may explain why sleep of older people is characterized by the few profitable and a number of detrimental features (i.e., a better ability to cope with daytime sleepiness and sleep loss vs. difficulty of falling asleep, decreased total nighttime sleep, "lightened" and fragmentized sleep, unwanted early morning awakenings, etc.).

Keywords: Circadian rhythms, EEG spectrum, normal aging process, principal component analysis, sleep homeostasis, sleep disturbances, sleep-wake regulation, slow-wave activity.

INTRODUCTION

Sleep quality is an important aspect of successful aging. Epidemiological studies indicate that even the healthy aging process is often accompanied by profound disruptions of individual sleep-wake cycles [1-5]. It has been estimated that approximately every second older person in the United States of America experiences sleep-wake disturbances [1]. They usually include unsatisfactory daytime alertness, undesired daytime sleepiness, too frequent daytime napping, difficulty of falling asleep, insufficient duration of nighttime sleep, unsatisfactory nocturnal sleep quality, disturbed or "light" sleep, too frequent nighttime awakenings, and unwanted early morning awakenings [1-5]. These epidemiological findings were corroborated by data from clinical and experimental research. Several polysomnographic studies revealed such age-related changes in objective characteristics of the sleep-wake cycle, such as increased daytime napping, longer sleep latency, decreased total nocturnal sleep time, reduced threshold for awakenings from sleep, fragmentation of sleep with multiple awakenings, advanced circadian phase of sleep offset and onset, shortened rapid-eye-movement

(REM) sleep latency, shortened ultradian sleep cycles, increased "light" sleep (stages 1 and 2 of non-REM sleep), decreased "deep" or slow wave sleep (stages 3 and 4 of non-REM sleep), decreased REM sleep, increased REM sleep in the earlier parts of night, and decreased REM sleep in the later parts of night [6-12]. It was also noted that some of these changes in objective sleep characteristics were detected even in healthy, carefully screened, and non-complaining older individuals [2, 4, 13]. On the other hand, some reports indicate that increasing age is not always associated with worsening of objective sleep characteristics, at least, in the female subpopulation [14]. Moreover, the process of normal aging can bring some advantages to older people living in modern 24-hour societies. For instance, they may be less sleepy during daytime and they better tolerate sleep deprivation, when compared to younger people [15-18].

Age-related sleep disturbances are considered to relate to ontogenetic changes of physiological systems which regulate the sleep-wake cycle. However, the exact nature of these changes is not clearly understood, and there is still much to learn about regulatory age-related mechanisms underlying normal and pathological sleep [2, 5]. The present report is the first attempt to apply the procedure of principal component structuring to the sleep electroencephalographic (EEG)

*Address correspondence to this author at the 11, Nipkowstr., 12489 Berlin, Germany; Tel: 0049-30-61290031; Fax: 0049-30-53674643; E-mail: putilov@ngs.ru

spectra to separate influences of the opponent sleep- and wake-promoting processes on the observed age-related modifications of the objective sleep indexes.

Theoretically, such an attempt might be related to the widely known conceptualization of the sleep-wake regulation - the two-process model [19] – that postulates two fundamental regulatory processes: a sleep-wake-dependent homeostatic process and a circadian process. The first process regulates a dynamic balance between the duration of prior wakefulness, and duration and intensity of the consecutive sleep episode. The second process is mostly unaffected by prior history of sleep and represents the circadian regulation of sleep-wakefulness across 24-h cycles [19, 20]. Accordingly, the effects of aging on objective sleep quality can be attributed to i) a reduced homeostatic drive, ii) a reduced circadian drive, and iii) an altered interaction of the homeostatic and circadian drives [21]. The reported results of the differential contribution of circadian and homeostatic drives to age-related changes in sleep architecture appear to be inconclusive. Although some of such changes can be associated with a circadian deregulation (i.e., the age-related weakening of the circadian drive [21, 22]), there is no clear agreement among sleep researchers whether the homeostatic drive is altered in older healthy adults. Some reports pointed on a less pronounced sleep homeostasis in older people, as indexed by a reduction of slow wave sleep and sleep spindles [16-27]. These results were confirmed by several studies utilizing EEG slow-wave activity (i.e., 0.5-4.5 Hz) which was proposed to be the major physiological marker of the homeostatic process [19, 20]. Such studies, suggested that the decrease of both, EEG slow-wave activity and slow wave sleep are the most obvious age-related modifications of the sleep EEG [10, 28]. An attenuated response of slow-wave activity to prolonged wakefulness (i.e., insufficient increase of EEG power density in the slow-wave range) appears to be the most common characteristic of the EEG signal recorded during recovery sleep in older study participants [10, 22, 28-30]. By contrast, some experimental studies showed no general change of the homeostatic sleep drive in older volunteers, especially in experiments to shortened duration of wakefulness [26, 27, 31, 32]. Additionally, extension or reduction of prior wakefulness led to topographical differences in EEG slow-wave and sleep spindle activity between young and older study participants during recovery sleep [24, 25, 27, 33, 34]. Older study participants showed an attenuated frontal predominance of EEG slow-wave activity during recovery sleep, a result which is already present in middle aged adults [35]. Taken together, it seems that the sleep homeostat is still operational in older people, but may be less pronounced and brain region-dependent [21, 26, 27, 33, 34].

Our previous results suggested the reduction of slow wave and REM sleep in naps of healthy older participants compared to healthy younger participants, but there were no significant age-related changes in most other objective sleep measures, such as sleep latency, total sleep time, and sleep efficiency [27]. Therefore, our present analysis focused on a detailed examination of subtle but reliably replicated differences between young and 'very healthy' older volunteers on

the objective characteristics of sleep during multiple nap episodes, occurring under low homeostatic sleep pressure. Since these episodes were scheduled at different clock times throughout a 40-h nap protocol [26, 27], such a study design allowed the evaluation of age-related changes in the sleep process per se by averaging out potential homeostatic influences on sleep-wake regulation (i.e., by calculating individual means for multiple napping episodes).

In the two-process model, the kinetics of EEG slow-wave activity was associated with the homeostatic process and explained by the influence of the so-called "sleep drive" [19]. We showed in several publications [35-40] that "signatures" of, at least, two distinct underlying processes of homeostatic sleep-wake regulation can be separated by examining the pattern of inter-correlations among the frequencies of the EEG spectrum, and, by extracting orthogonal, (i.e., uncorrelated and independent) principal components. Particularly, it was demonstrated that the time courses of scores in the two largest principal components tended to oppose each other at the transitions between wakefulness and sleep. It seems that the time course of the 1st (largest) principal component score reflects the regulation of sleep need by a sleep drive (promoter) that was acknowledged by the two-process model [19]. However, the time course of the 2nd principal component score is more likely to be associated with a wake drive (promoter) to regulate wake/sleep pressure. It was not directly addressed by the two-process model because, (as we noted [37-40]), the classical EEG slow-wave activity index strongly correlates with the difference between scores from the 1st and 2nd principal components. It can represent a dynamic balance between the competitive sleep and wakefulness drives i.e. between sleep need and wake/sleep pressure, respectively [35-40].

Consequently, our major hypothesis was that an age-related decrease of EEG slow-wave activity might reflect the changes either only in one of two, or in both opponent processes of homeostatic sleep regulation. If the decrease of EEG slow-wave activity is a result of a weaker sleep drive with aging, one can find that an attenuated buildup of the 1st principal component score during each nap, irrespective of circadian phase. If it is a consequence of a stronger wake drive, one can find during each nap that the 2nd score is elevated, rather than attenuated. Finally, if both sleep drives are weaker and the wake drive becomes stronger with advancing age, one can find that an attenuated buildup of the 1st score is combined with an insufficient decline of the 2nd score. If the wake drive strengthening will be supported by experimental data, this can explain a possible advantageous traits of the sleep-wake behavior in older people: their better ability to cope with daytime sleepiness and sleep loss. On the other hand, it can also explain the numerous signs of worsening of sleep quality in the process of pathological and healthy aging.

MATERIALS AND METHODS

The study design and the methods of sleep data collection have been detailed in previous publications [25-27]. Briefly, the study participants were 15 older and 16 younger healthy volunteers (57-74 and 20-31 years, respec-

tively). All participants gave written informed consent. The study protocol, the screening questionnaires and consent form were approved by the local Ethical Committee and conformed with the Declaration of Helsinki. On the baseline week prior to admission to the laboratory, participants were asked to abstain from excessive caffeine and alcohol consumption and to keep a regular sleep-wake schedule. Compliance was verified by sleep logs and ambulatory activity measurements (wrist activity monitor, Cambridge Neurotechnology Ltd, UK). In the laboratory, two baseline sleep episodes were followed by 225-min sleep-wake cycles (75 minutes in bed, 150 minutes awake) for 40 hours. Participants remained in dim light conditions (<8 lux during wakefulness and 0 lux during 75-min sleep opportunities), under constant semirecumbent posture position in bed with regular meals.

The objective characteristics of sleep during eleven 75-min sleep opportunities were previously reported for the groups of older and younger participants in Münch *et al*. [26, 27] and Knoblauch *et al*. [25]. Sleep stages were visually scored according to the standard criteria per 20-s epochs [41]. For each 75-min sleep opportunity, spectral EEG power density was determined for all epochs classified as non-rapid eye movement (non-REM) sleep. Spectral analysis was performed on four EEG midline derivations, frontal (Fz), central (Cz), parietal (Pz), and occipital (Oz), referenced against linked mastoids, A1 and A2. A Fast Fourier transform with 10% cosine 1-s window resulted in a 0.25 Hz bin resolution. After selection of artifact-free epochs, up to five 4-s epochs were averaged to maintain correspondence with the 20-s sleep scoring interval. For the current analysis, data from 20-s epochs were further averaged over 15-min intervals or

quarters. Thus, each non-REM containing nap was represented by a sequence of up to 5 quarters. Sixty four absolute power values for frequencies from 0.5 to 16.5 Hz were log-transformed and subjected to principal component analysis. In addition, these log-transformed values were binned in frequency bands corresponding to slow-wave activity (SWA, 0.5-4.5 Hz), theta-activity (4.75-7.5 Hz), alpha-activity (7.75-12 Hz), and low and high spindle frequency activity (LSFA, 12.25-13.25 Hz, and HSFA, 13.5-16.5 Hz, respectively).

The SPSS statistical software package was used for all statistical analyses (SPSS Inc., Chicago, IL, version 17.0). Principal component analysis was run to uncover the pattern of inter-correlations among the 64 log-transformed power density values. The four largest components (1st – 4th) were extracted to decompose each set of 64 powers into just four principal component scores (Table **1**). The meaning of principal components was interpreted by plotting the loadings of the 64 powers on each component as a function of separate frequency bands from 0.5 to 16.5 Hz (Fig. **1**). To calculate a score on each of the four components, the 64 original values were optimally weighted according their loadings (Fig. **1**) and then summed. The scores were calculated separately for 4 subsets of spectra from Fz, Cz, Pz, and Oz (bottom part of Table **1**).

Fig. (**2**) illustrates the averaged time courses of scores on each of four principal components (A-D). Figs. (**3**) and (**4**) show the time courses of pairwise combinations of principal component scores, and Fig. (**5**) provides a possibility to compare these time courses with the time courses of the conventional EEG indexes, such as log-transformed powers for separate frequency ranges.

Table 1. Total Variance Explained by the four Largest Principal Components of the EEG Spectrum

Principal components	Eigenvalue				% of Variance				Cumulative %			
	1st	2nd	3rd	4th	1st	2nd	3rd	4th	1st	2nd	3rd	4th
The whole sample	41.1	8.6	4.5	2.6	64.2	13.4	7.1	4.1	64.2	77.5	84.6	88.7
Two groups of study participants:												
Older (Fig. **1A**, left)	44.1	6.7	4.2	2.3	68.9	10.5	6.6	3.7	68.9	79.3	85.9	89.6
Younger (Fig. **1A**, right)	37.5	10.7	5.3	3.1	58.6	16.7	8.3	4.9	58.6	75.3	83.6	88.5
Four separate derivations:												
Fz (Fig. **1B**, left)	39.7	8.4	5.3	2.9	62.0	13.1	8.2	4.5	62.0	75.1	83.4	87.9
Cz (Fig. **1B**, right)	39.2	9.2	4.7	2.9	61.2	14.4	7.4	4.6	61.2	75.6	83.0	87.6
Pz (Fig. **1C**, left)	37.8	11.1	4.8	2.3	59.1	17.4	7.5	3.5	59.1	76.5	84.0	87.5
Oz (Fig.**1C**, right)	38.7	11.9	4.4	1.9	60.4	18.5	6.9	2.9	60.4	78.9	85.8	88.8

Notes. 1st, 2nd, 3rd, and 4th: the four largest principal components of the EEG spectra were calculated from 64 log-transformed spectral power values in the frequency range from 0.5 to 16.5 Hz. Principal component analysis was performed on the whole data sample (5440 EEG spectra obtained by averaging across five 15-min intervals of each nap), on two subsets of older and younger study participants (2696 and 2744 EEG spectra, respectively), and on four subsets for Fz, Cz, Pz, and Oz derivations (1360 EEG spectra each).
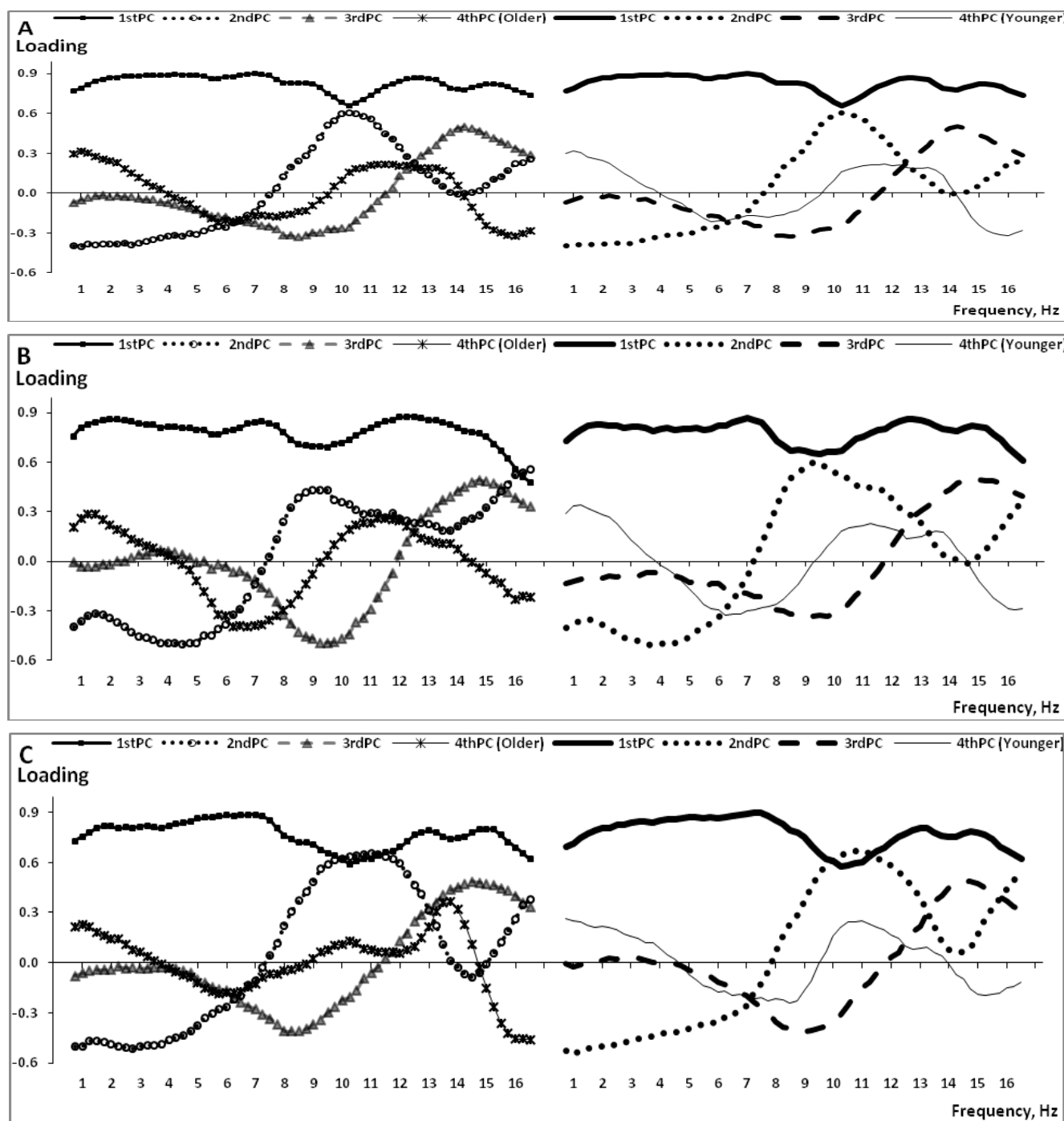
Fig. (1). Loading spectra of the four largest principal components of the EEG spectrum. The loading spectra (1st PC, 2nd PC, 3rd PC, and 4th PC) were calculated after dividing the whole data set into two subsets of data for older and younger participants (A: left and right plots, re-spectively), and into four subsets of data from for frontal, central, parietal, and occipital derivations (B: Fz, left plots, Cz, right plots; C: Pz, left plots, and Oz, right plots). Loading represents the correlation between a component and a power (because this is a correlation, a possible loading can vary in the range from -1 to +1). See also notes to Table **1**.
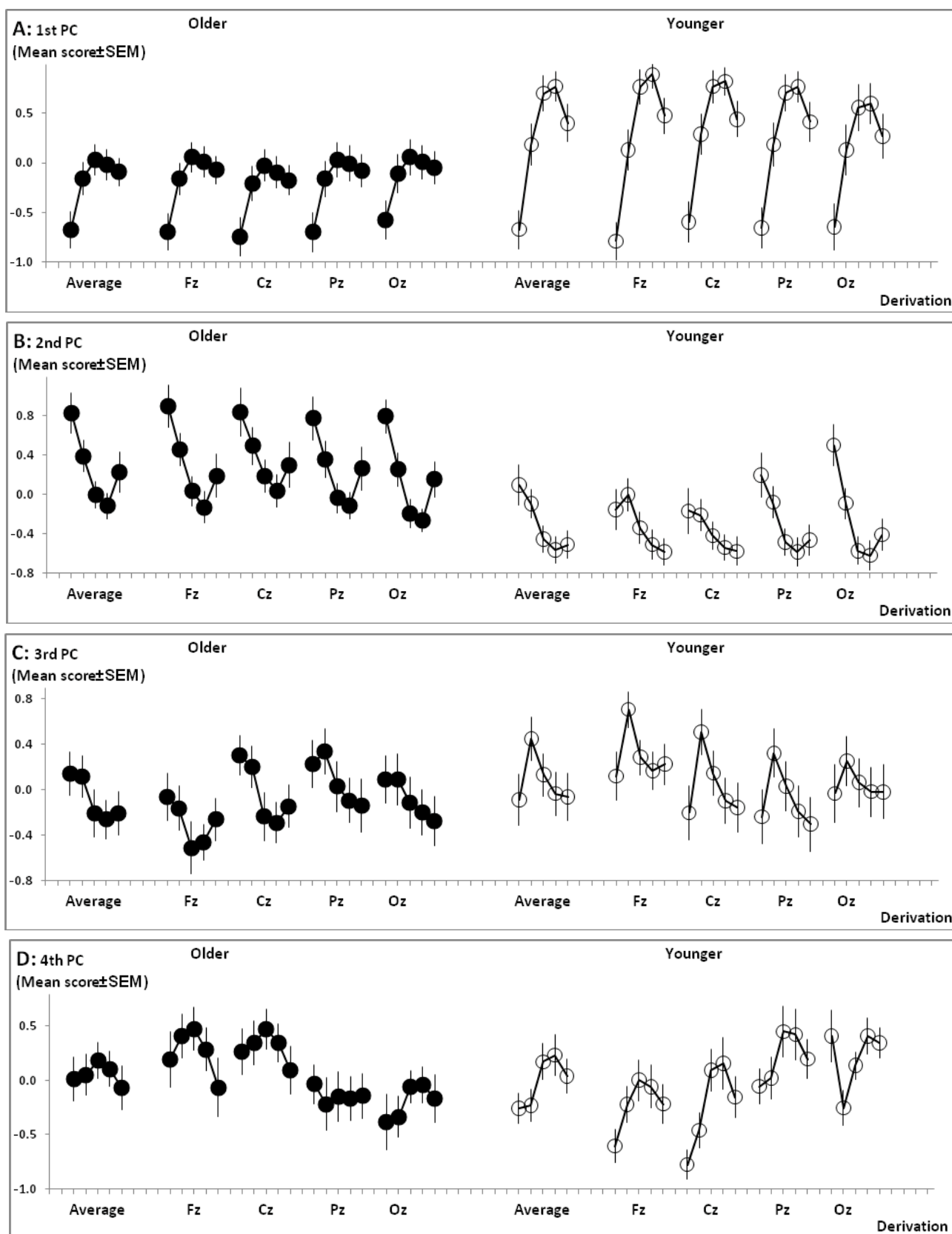
Fig. (2). Within-nap time courses of the four largest principal components scores. Time courses of scores of the four largest principal components of the EEG spectra in a range from 0.5 to 16.5 Hz (A-D: 1st, 2nd, 3rd, and 4th PC, respectively). Right and left side plots: scores were obtained by averaging over naps within each participant, and then over the groups of older and younger participants, respectively. Data represent means ± SEM for frontal, central, parietal, and occipital derivations (Fz, Cz, Pz, and Oz, respectively), and all 4 derivations (Average). See also the statistical results and notes to Table **2**.
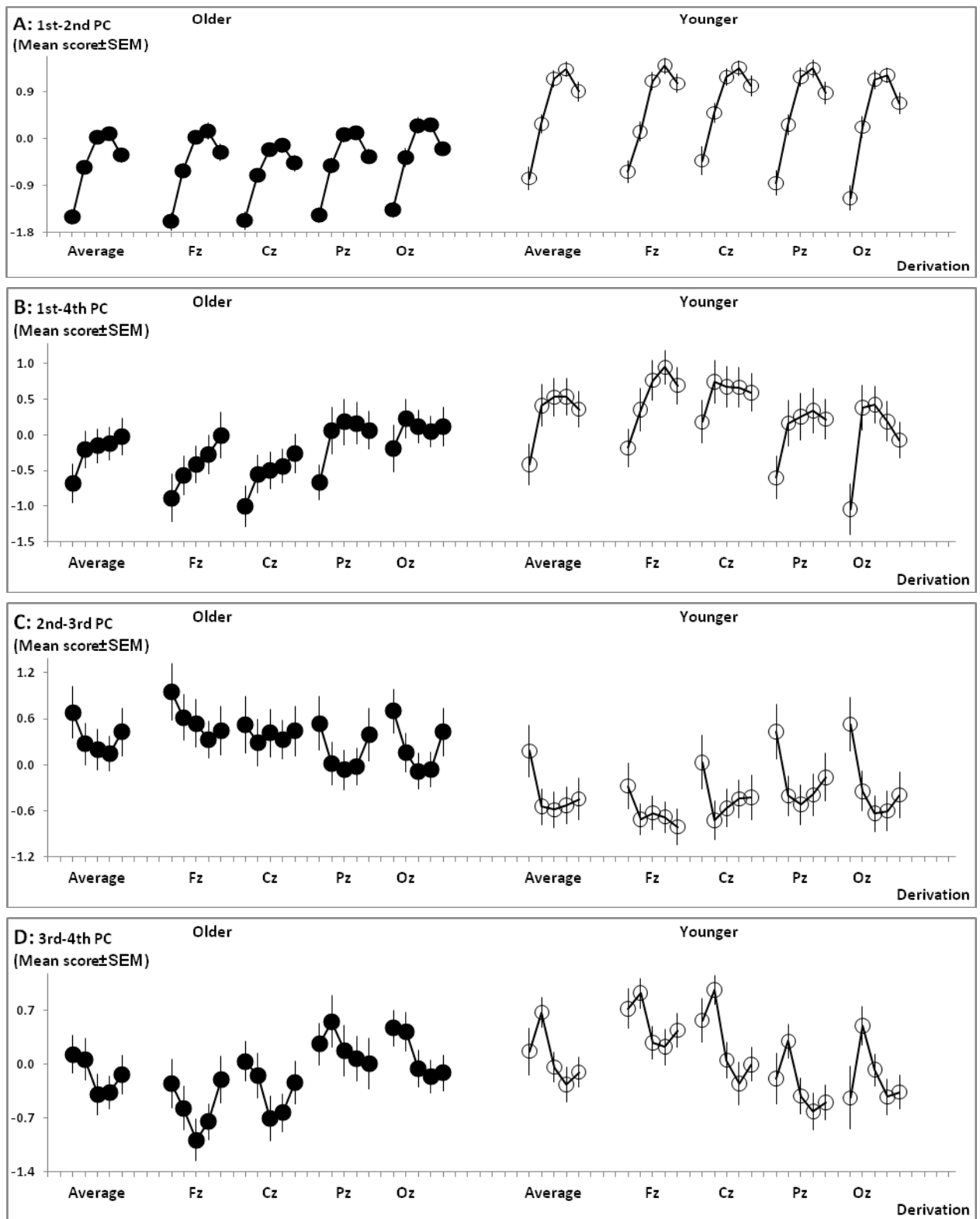
Fig. (3). Within-nap time courses of the differences between principal components scores. Time courses were obtained by subtracting one principal component score from another (A-D: 1st-2nd PC, 1st-4th PC, etc.). See also the legend to Fig. (**2**).
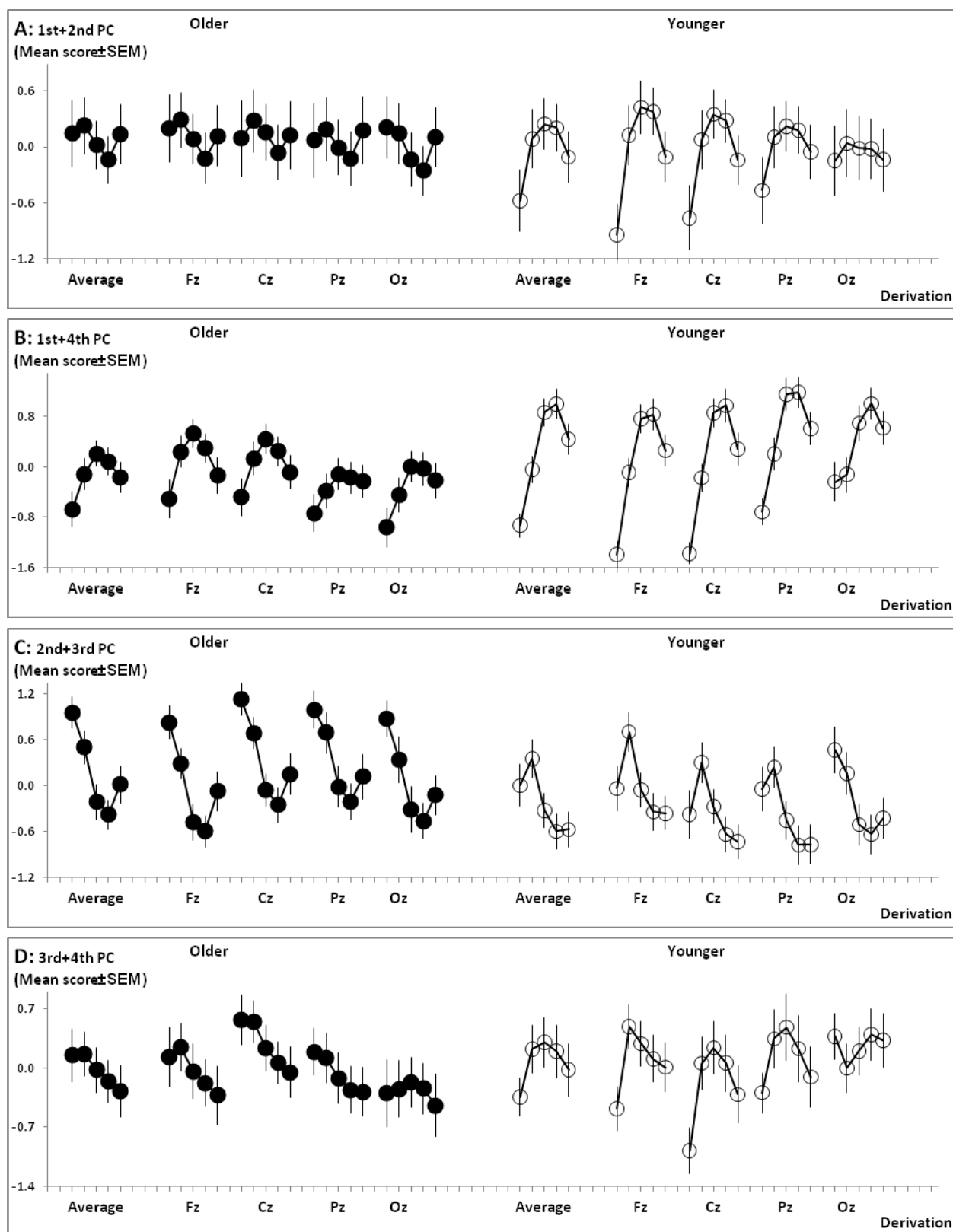
Fig. (4). Within-nap time courses of the sums of principal components scores. Time courses were obtained by summing up principal component scores (A-D: $1^{st}+2^{nd}$ PC, $1^{st}+4^{th}$ PC, etc.). See also the legend to Fig. (**2**).
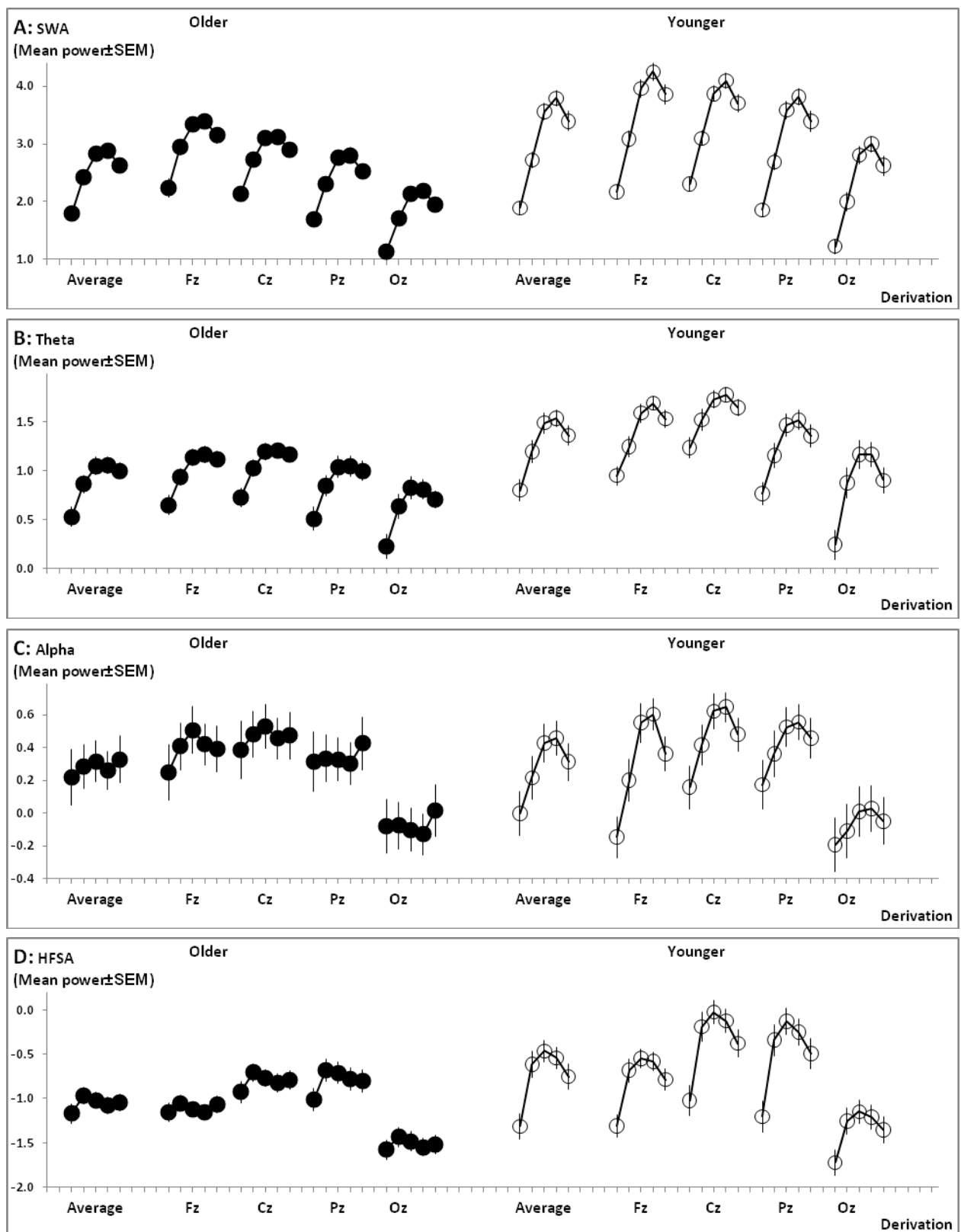
Fig. (5). Within-nap time courses of conventional EEG indexes. Time courses represent the spectral powers in four frequency ranges: slow-wave activity (A: SWA), theta frequency activity (B), alpha frequency activity (C), and high spindle frequency activity (D: HSFA). The spectral powers for separate frequency ranges were averaged within 15-min intervals, log-transformed, and averaged within and then across older and younger participants. See also the legend to Fig. (**2**).

In order to evaluate age differences within time courses of 8 EEG indexes, we performed two-way repeated measure ANOVAs (rANOVAs) with one repeated measure ("Quarter" or 15-min interval of 75-min nap: from 1st to 5th), and one independent factor ("Group": older and younger participants). The spatial differences (Table **2**) were evaluated with three-way rANOVAs by adding another repeated measure ("Derivation": from Fz to Oz).

Besides, the significance of the effect of nap timing on the age-associated differences in mean levels and within-nap variation in the EEG indexes were tested using three-way ANOVAs with three independent factors ("Group", "Quarter", and "Nap": from 1st to 11th).

Additionally, Pearson coefficients of correlation were calculated to test, whether the within-nap time courses of the EEG indexes (as shown in (Figs. **2-5**) are in sync in the groups of older and younger participants. A pattern of correlation suggests that they are shifted one relative to another.

RESULTS

Variance Explained by four Principal Components

Principal component analysis, based on the inter-correlations among the 64 log-transformed power densities (0.5 Hz -16.5 Hz) which yielded the first four eigenvalues higher than 1 (Table **1**). Particularly, almost 90% of the total variance was collectively explained by the 1st, 2nd, 3rd, and 4th principal components. Inspection of the patterns of loadings (i.e., loading spectra) indicated that the shapes of the principal components were roughly identical either for the entire data set and for the two separate groups of participants as well as for the four derivations (Fig. **1** and Table **1**). The 1st

Table 2. F-values from the Results of Two- and Three-way rANOVAs of the EEG Indexes

Fac-tor	Deri-vation	Index df	Principal component score				Conventional EEG index			
			1st	2nd	3rd	4th	SWA	Theta	Alpha	HSFA
2- and 3-way rANOVA: Factor "Quarter" (1)										
1	Average	4	62.9[***]	29.4[***]	7.0[***]	8.6[***]	86.9[***]	65.0[***]	10.6[***]	22.4[***]
1	Fz	4	59.6[***]	24.0[***]	5.8[**]	7.0[**]	86.3[***]	59.9[***]	24.0[***]	17.4[***]
1	Cz	4	53.5[***]	14.8[***]	8.9[***]	13.7[***]	73.9[***]	48.3[***]	12.2[***]	23.0[***]
1	Pz	4	67.7[***]	25.5[***]	9.1[***]	3.1[*]	83.6[***]	63.7[***]	5.5[**]	28.5[***]
1	Oz	4	63.2[***]	46.8[***]	3.1[*]	8.0[**]	96.7[***]	64.7[***]	1.9	13.3[***]
2- and 3-way rANOVA: Factor "Group" (2)										
2	Average	1	4.2	7.9[**]	0.4	0.1	14.9[**]	9.2[**]	0.0	4.4
2	Fz	1	4.9[*]	7.5[*]	6.5[*]	3.4	7.9[**]	12.9[**]	0.2	6.1[*]
2	Cz	1	7.0[*]	10.4[**]	0.1	4.5[*]	19.4[***]	20.4[***]	0.0	7.4[*]
2	Pz	1	3.5	5.9[*]	0.3	1.6	19.3[***]	6.1[***]	0.2	2.8
2	Oz	1	1.3	3.9	0.2	3.0	9.9[**]	1.9	0.0	1.1
2- and 3-way rANOVA: Interaction between factors "Quarter" (1) and "Group" (2)										
1x2	Average	4	7.9[**]	1.6	3.1[*]	4.2[**]	7.3[**]	1.7	5.9[**]	11.1[***]
1x2	Fz	4	9.1[***]	6.0[**]	4.0[*]	3.1[*]	7.9[**]	2.2	8.1[***]	14.5[***]
1x2	Cz	4	6.5[**]	2.2	6.0[**]	9.3[***]	6.6[**]	0.3	5.4[**]	11.9[***]
1x2	Pz	4	7.4[**]	0.9	1.8	4.5[**]	7.0[**]	1.7	4.8[*]	8.8[***]
1x2	Oz	4	7.2[**]	0.6	1.1	4.8[*]	6.8[**]	3.1	3.0	7.2[**]
3-way rANOVA: Interactions between factors "Quarter" (1), "Group" (2), and "Derivation" (3)										
1x3	4 (Fz, Cz, Pz, and Oz)	12	11.8[***]	18.0[***]	5.2[***]	6.9[***]	321.9[***]	56.2[***]	48.1[***]	106.5[***]
2x3		3	1.2	2.7	9.8[***]	15.6[***]	11.9[***]	18.1[***]	20.1[***]	18.8[***]
1x2x3		12	4.1[**]	7.8[***]	4.0[**]	6.7[***]	2.5	3.2	0.9	2.4

Notes. Level of significance for F-values from the results of two- and three-way rANOVAs: [***] (p<0.001), [**] (p<0.01), [*] (p<0.05). 1st, 2nd, 3rd, and 4th: Scores on the four largest principal components of the EEG spectra in a range from 0.5 to 16.5 Hz were calculated separately for four derivations (bottom part of Table **1** and Fig. **1B** and **C**). SWA, Theta, Alpha, and HSFA: Slow-wave activity, theta frequency activity, alpha frequency activity, and high spindle frequency activity. The EEG indexes for nonREM sleep epochs were averaged within 15-min intervals and within participants. "Quarter" (1): a sequence of five 15-min intervals containing nonREM sleep epochs. "Group" (2): two groups (15 older and 16 younger participants). "Derivation" (3): four derivations (Fz, Cz, Pz, and Oz). Average: Mean value for four derivations. See also the notes to Table **1** and Figs. **2-5**.

principal component (1st PC) of the EEG spectral variance was positively associated with the amplitude of oscillations of the EEG signal across all frequency bands. The 2nd component (2nd PC) was characterized by low amplitudes in the low frequency bands and high amplitudes in the high frequency bands. The 3rd component (3rd PC) was associated with damped EEG oscillations in the center of the analyzed interval of frequencies and with amplified oscillations in the faster frequency bands. The 4th component (4th PC) showed two areas of high amplitudes in the beginning and two areas of low amplitudes at the end of each of the two halves of the analyzed interval of frequencies. The areas of positive loadings roughly corresponded to the oscillations of the EEG signal in the slow wave and alpha ranges.

Rationale for Averaging Data Over All Successful Naps

According to the results of the three-way ANOVAs, only non-significant interactions were found between factors "Quarter" and "Nap", as well between factors "Quarter", "Group", and "Nap" (for any EEG index p>0.262 and p>0.740, respectively). Such findings provided the rationale for calculating individual mean values for all successful nap attempts of each study participant that is expected to average out the circadian influence on the sleep EEG spectrum. Consequently, further analysis was focused on the differences between the two groups and patterns of within-nap variation in the EEG.

Scores on the 1st and 2nd Principal Components

Fig. (**2**) illustrates the within-nap time courses of scores from principal components. Each time course consists of a sequence of 5 quarters (15-min time intervals). Five plots for older and 5 plots for younger groups are shown on the left and right sides, respectively. The first plot was obtained by averaging across all derivations and the next 4 plots represent different derivations (Fz to Oz). The comparison of the curves calculated for 1st and 2nd scores (Fig. **2A** and **B**) showed that the components always tended to oppose each other in the course of the ultradian sleep cycle. The course of the 1st score followed a reversed U-shape or a reversed L-shape wave-form (Fig. **2A**). In contrast, the courses of the 2nd score followed a U-shape or an L-shape wave-form (Fig. **2B**).

Table **2** summarizes the statistical results of the differences between the two groups on the levels and patterns of within-nap variations in these scores and other EEG indexes. This table contains F-ratios from two- or three-way rANOVAs with the repeated measure "Quarter" (1: a sequence of five 15-min intervals containing nonREM sleep epochs), and the independent factor "Group" (2: the groups of 15 older and 16 younger participants). The 3rd factor in three-way rANOVAs was "Derivation" (3: four derivations from Fz to Oz). The results shown in Table **2** consistently confirmed significance of the within-nap variation in the scores on the 1st and 2nd principal components (Fig. **2A** and **B**) as indicated by the levels of significance of main effects of the repeated measure "Quarter" (p<0.001 for all). The 1st principal component score was significantly lower in the older participants compared to the younger participants in more anterior derivations (Fz and Cz). The 2nd score was significantly higher in

the older participants in Fz and Cz and in Pz. In fact, similarity between the age groups was noted only for the 1st score for the first quarter (Fig. **2A**). This result suggests that younger participants showed a higher amplitude of the within-nap variation in the 1st but not in the 2nd score.

Moreover, "Quarter" as a repeated measure and "Group" as an independent factor revealed a significant interaction effect for the 1st score (p<0.001 for data from the frontal derivation, and p<0.01 for data from other derivations). Such an interaction was mostly non-significant for the 2nd score. The exception was the interaction for data from frontal derivations (Table **2**; Fig. **2**).

Scores on the 3rd and 4th Principal Components

Fig. (**2**) also shows, similar to the scores of the first pair of principal components (Fig. **2A** and **B**) such the scores on the second pair opposed each other such that the 3rd score tended to go up, while the 4th score went down, and the 4th score, in turn, tended to go up while the 3rd score went down (Fig. **2C** and **D**). The results given in Table **2** indicate that the within-nap variations in the 3rd and 4th scores always reached, at least, the 95% level of significance. The differences between groups from mean scores were non-significant with the exception of scores from Fz and Cz for the 3rd and 4th principal components (p<0.05).

The two- and three-way rANOVAs with the repeated measure "Quarter" and independent factor "Group" yielded a significant interaction. It was most pronounced in the central derivation (p<0.01 and p<0.001 for the 3rd and 4th score, respectively). We additionally found that the curves for the older participants often better correlated with the curves of the younger participants with a delay of 15min.

Moreover, three-way rANOVAs of the principal component scores yielded the significant interactions both between all three factors, and between the factors "Quarter" and "Derivation". The interaction between the factors "Group" and "Derivation" was significant for the second pair of scores that is in contrast to the insignificant results for the first pair of scores (Table **2**). Compared to the older participants, the younger participants had higher 3rd scores and lower 4th scores in more anterior derivations, but such differences were not evident in more posterior derivations. Since the scores were separately calculated for each of four derivations, the main effects of "Derivation" are not reported in Table **2**.

Combinations of Scores on the two Largest Principal Components and Conventional EEG Indexes

Fig. (**3**) reveals that, after subtracting the 2nd score from the 1st score, the differences between the groups on average and in amplitude in the within-nap variation became even more pronounced (Fig. **3A**). Fig. (**4**) illustrates that, after summing these scores, the amplitude of within-nap variations was considerably lowered in the older participants, whereas the curves still exhibited the reversed U-shape or reversed L-shape wave-forms in the younger participants (Fig. **4A**).

Fig. (**5**) documents the within-nap time courses of the conventional spectral EEG indexes. For the younger participants, all wave-forms (Fig. **5A-D**) roughly resembled the wave-form of the 1st score (Fig. **2A**). In contrast, the time

courses of the older participants showed more remarkable variability. As in the younger participants, the pattern of within-nap variations in the low frequency powers (i.e., in delta and theta bands) resembled the time course of the 1st score. In contrast, such reversed U-shape or reversed L-shape wave-forms were not evident for the high frequency powers.

Noteworthy, the time courses of the conventional spectral EEG indexes (Fig. **5**) can be easily predicted from the loading patterns of the 1st and 2nd principal components (Fig. **1**), which collectively accounted for approximately 75% of the total variance in the EEG spectrum (Table **1**). Both of these components were characterized by positive loadings on the high frequency range (i.e., alpha and sigma bands). In contrast, the 1st component loaded positively and the 2nd component loaded negatively in the low frequency range (i.e., delta and theta bands). Therefore, high frequency activity can be regarded as main effect of summation in the two largest principal components of the EEG spectrum. Low frequency activity can reflect their differential effect. Consequently, a close resemblance was observed between the time courses of the sum of the two scores (Fig. **4A**) and the high frequency powers, i.e., alpha power (Fig. **5C** and **D**), and between the time courses of the difference between the two scores (Fig. **3A**) and the low frequency powers, i.e., delta power (Fig. **5A** and **B**).

Table **2** also documents the results of two- and three-way ANOVAs of the conventional EEG indexes. Similar to the results of rANOVAs of the time courses of principal component scores, these rANOVAs revealed highly significant main effects of the within-subjects factor "Quarter". The main effect of "Group" was significant for all but alpha frequency ranges. With the exception of theta power, there were also significant interactions between these two factors. Three-way rANOVAs yielded significant interactions of the factor "Group" with two other factors, but, in contrast to the rANOVAs' results on principal component scores, the triple interactions between these factors were non-significant (Table **2**).

Levels of Significance for Results Obtained from Anterior and Posterior Derivations

Finally, we noted that both main effects and interactions reached the level of significance more often in the results obtained from the frontal and central derivations rather than from the occipital derivations (Table **2**).

DISCUSSION

In order to shed light on the chronoregulatory mechanisms underlying advantageous and disadvantageous features of sleep in older people, we examined those age-related differences in the EEG indicators of the opponent sleep- and wake-promoting processes that can be detected irrespective of circadian timing of nap episode and in the absence of sleep restriction. To our knowledge, this is the first comparison of younger and older individuals conducted by means of the principal component analysis of non-REM sleep EEG spectra. We aimed at testing a possibility that only one or both opponent processes of homeostatic sleep regulation contribute to age-related differences in the objective sleep measures. The results indicate that such analysis can provide a deeper insight into the regulatory processes underlying both optimal sleep of older people and their predisposition to develop certain disturbances of their sleep-wake cycle.

For the present analysis, we used data on multiple naps showing that healthy older participants compared to healthy younger participants spent less time in slow wave and REM sleep, but the two groups did not differ on most other objective sleep measures, such as sleep latency, total sleep time, and sleep efficiency during the nap protocol [27]. The finding of only minor changes in sleep architecture in healthy elderly people is not surprising, when facing epidemiological investigations (see Introduction). These results not only show the increase in the rate of sleep problems, but also indicate the absence of complaints about such problems in every second elderly person, and, thus, they support the notion that advanced age does not necessary result in disturbed or unsatisfied sleep [2]. The present comparisons of the mean levels and within-nap time courses of scores from the largest principal components of the EEG spectra in older and younger study participants revealed several significant age-related changes. They included a decrease in the 1st score in the frontal derivation and an increase in the 2nd principal component score in the majority of derivations (Table **2** and Fig. **1A** and **B**), as well as an advanced shift of the phases of the first ultradian cycle of the 2nd, 3rd, and 4th principal component scores (Fig. **1B-D**).

In general, the present results corroborate the idea of the opponent nature of sleep-wake regulating processes that can be revealed by the time courses of scores from the four largest principal components of the EEG spectrum [35-40]. It seems that the time courses can be regarded as the EEG "signatures" of these underlying processes. For instance, the time courses of the 1st and 2nd scores appear to reflect the sleep- and wake-promoting processes, respectively. Moreover, the present results confirmed that, in terms of the principal component structure of EEG spectrum, the measures of EEG slow-wave activity can be indicators of the balance between the opponent sleep and wake drives (promoters). Specifically, we confirmed an earlier made observation [37-40] that these measures are almost identical to the difference between the 1st and 2nd principal component scores (Fig. **3A** and **5A**), and that the within-nap variations of these scores can be studied to separate the kinetic features of the sleep and wake drives. Consequently, such separation of the markers of the two drives provides a good tool to test our hypotheses that the age-related decrease of slow-wave activity might be regarded as reflecting either 1) weakening of the sleep drive associated with the 1st score or 2) strengthening of the wake drive associated with the 2nd score or 3) both weakening of the sleep drive and strengthening of the wake drive. The present findings allowed the conclusion that, with advanced age, the 1st score undergoes a significant reduction while the 2nd score significantly increases (Fig. **2 A** and **B**; Table **2**), and, hence, both drives are likely to be involved in the age-related decline of EEG slow-wave activity and slow-wave sleep.

Particularly, the observed time course of the 1st score (Fig. **2A**) suggests that the strength of sleep drive in older participants can be similar to that in younger participants

only in the very beginning of the non-REM sleep (the 1st quarter). This similarity may indicate that the two groups had also similar sleep latency. However, with further progression of sleep (quarters 2-5)m a significant reduction of the strength of sleep drive was observed in the older participants, when as compared to younger participants (Fig. **2A**). Such a reduction can reflect the predisposition of older people to an attenuated response of their homeostatic sleep drive to sleep deprivation. The enlargement of the 2nd principal component score in older participants can be regarded as evidence for strengthening of their wake drive (Fig. **2B** and Table **2**). One can expect that such strengthening might predispose both to certain night sleep disturbances and to certain beneficial effects during wake time. More specifically, the absence of age-related differences on the 1st score in the beginning of sleep in combination with the age-related increase of 2nd score can explain the ability of older healthy people to better cope with the effects of sleep deprivation and daytime sleepiness which was reliably documented in several recent reports [16-18, 42].

It was reported that, chronologically, a decrease of slow wave sleep at the cost of an increase of "light" non-REM sleep signifies an earlier stage of sleep 'aging', while a later aging stage is characterized by an increase in awakening at the expense of total sleep time [8]. It appears that the present findings on the first pair of principal components can explain such chronology of age-related changes in sleep architecture. These changes can be viewed as a single process of the gradual change of the wake and sleep drives (strengthening and weakening, respectively). Due to small changes in the strength of both drives, the changes in sleep architecture at the very first step on the way toward the age-related sleep disturbances can be noted only in a form of significant decrease of "deep" non-REM sleep that is, however, compensated by an increase of "light" non-REM sleep. The next naturally expected step on this way (those corresponding to the late rather than early aging process) can be associated with a significant lower threshold for arousal from sleep, a decrease of total sleep time, and an increase of time spent awake at the expense of different sub-states of sleep. In other words, the decrease in "deep" sleep at the cost of the increase of "light" sleep can be regarded as the first link of the single chain of age-related sleep changes in sleep architecture. Only the following links of this chain represent serious sleep disturbances (i.e., those associated with the reduction of threshold for arousal from sleep, the fragmentation of sleep with multiple arousals, and the reduction of total sleep duration).

Since the 3rd and 4th principal components account for a rather small portion of the total variance in the EEG spectrum (Table **1**), the dynamic features of their scores cannot be detected by means of the traditional analysis of the EEG indexes in separate frequency ranges (i.e., compare Fig. **2C** and **D** with Fig. **5A-D**). Therefore, little is known yet about a possible role of the second pair of principal components in sleep-wake regulation, and the results from the 3rd and 4th scores need to be interpreted with caution. Nevertheless, the significant differences between age groups allow the suggestion that the changes in these scores also reflect the normal process of age-related sleep changes. The knowledge about age-related changes in these components can provide better

understanding of predisposition of older people to some sleep disturbances. For instance, the results point to a possibility of an age-related advance of the phases of ultradian oscillations of the 3rd and 4th scores (Fig. **2C** and **D**). Such an advance alone, or in combination with the above mentioned increase of the 1st score, and decrease of the 2nd score, could explain the shortening of REM latency, the increase of REM sleep in the earlier part of night, at the expense of REM sleep in the second part of night, and the shortening of the ultradian sleep cycle. Moreover, one can speculate about the possibility of a relationship between ultradian and circadian advances in older individuals, with earlier phases of ultradian sleep cycles and an earlier circadian timing of sleep onset and offset. Additionally, the unwanted early morning awakenings and earlier circadian sleep phase could be explained by the weakening of the sleep-promoting process and the strengthening of the wake-promoting process that can itself reveal as a shortened interval of a lower score on the 1st principal component combined with a shortened interval of rise on the 2nd principal component in the course of ultradian sleep cycles.

Thus, it seems that the documented changes in the four-component structure of the sleep EEG spectrum can help to identify the underlying chronoregulatory processes, responsible for the majority, if not all, changes in the objective characteristics of sleep in older people (i.e., those listed in the introduction section).

Although the frontal and occipital locations are known to be best suited for the determination of individualized frequency bands [43], anterior brain regions elicit more intense responses to changes in duration of prior wakefulness, and such topographical differences (i.e. the frontal predominance) are less pronounced in older study participants [24, 25, 27, 33, 34]. The present results are confirming these findings. Our results revealed that the difference between the two age groups from the principal component scores reached the level of statistical significance more often for the frontal and central derivations, than for the occipital derivation (Table **2**).

CONCLUSION

A better understanding of age-related effects in regulating sleep and wakefulness can be gained from the principal component analysis. Such analysis separates the influence of the sleep and wake drives and thus, can help in explaining the predisposition of older people to experience a number of sleep disturbances, on the one hand, and to possess few advantageous traits of their sleep-wake cycle, on the other hand. Most consistent age-related changes in sleep such as the decrease of EEG slow-wave activity can be interpreted as the result of the simultaneous weakening of the sleep drive and strengthening of the wake drive as indexed by the attenuated rise of the 1st principal component score and the attenuated fall of the 2nd score in the course of the first episode of non-REM sleep. Consequently, further changes in the strengths of these drives can lead to lower thresholds for arousal from sleep, the reduced night sleep, and the frequent awakenings during night sleep episodes. The age-related strengthening of the wake drive may explain the observation that older people despite their more fragmented night sleep,

better tolerate sleep deprivation and feel not sleepier during daytime' than younger people.

CONFLICT OF INTERESTS

The authors confirm that this article content has no conflict of interest.

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PATIENT'S CONSENT

Declared none.

REFERENCES

[1] Driscoll HC, Serody L, Patrick S, *et al*. Sleeping well, aging well: a descriptive and cross-sectional study of sleep in 'successful agers' 75 and older. Am J Geriatr Psychiatry 2008; 16(1): 74-82.

[2] Vitiello MV. Recent advances in understanding sleep and sleep disturbances in older adults: growing older does not mean sleeping poorly. Curr Direct Psychol Sci 2009; 18(6): 316-20.

[3] Foley DJ, Monjan AA, Brown SL, Simonsick EM, Wallace RB, Blazer DG. Sleep complaints among elderly persons: An epidemiologic study of three communities. Sleep 1995; 18: 425-32.

[4] Vitiello MV. Sleep disorders and aging: Understanding the causes. J Gerontol: Med Sci 1997; 52A(4): 189-91.

[5] Ohayon MM. Epidemiology of insomnia: what we know and what we still need to learn. Sleep Med Rev 2002; 6: 97-111.

[6] Webb W. Sleep in older persons: Sleep structures of 50- to 60-year-old men and women. J Gerontol 1982; 37(5): 581-6.

[7] Zepelin H, McDonald CS, Zammit GK. Effects of age on auditory awakening thresholds. J Gerontol 1984; 39(3): 581-6.

[8] Van Cauter E, Leproult R, Plat L. Age-related changes in slow wave sleep and REM sleep and relationship with growth hormone and cortisol levels in healthy men. JAMA 2000; 284: 861-8.

[9] Hoch C, Dew M, Reynolds C, Buysse D, Nowell P, Monk T. Longitudinal changes in diary- and laboratory-based sleep measures in healthy "old old" and "young-old" subjects: a three-year follow up. Sleep 1997; 20(3): 192-202.

[10] Carrier J, Land S, Buysse DJ, Kupfer DJ, Monk TH. The effects of age and gender on sleep EEG power spectral density in the middle years of life (20-60 years old). Psychophysiol 2001; 38: 232-42.

[11] Ohayon MM, Carskadon MA, Guilleminault C, Vitiello MV. Meta-analysis of quantitative sleep parameters from childhood to old age in healthy individuals: developing normative sleep values across the human lifespan. Sleep 2004; 27: 1255-73.

[12] Hita-Yañez E, Atienza M, Gil-Neciga E, Cantero JL. Disturbed sleep patterns in elders with mild cognitive impairment: the role of memory decline and ApoE ε4 genotype. Curr Alzheimer Res 2012; 9(3): 290-7.

[13] Vitiello MV, Larsen LH, Moe KE. Age-related sleep change: gender and estrogen effects on the subjective-objective sleep quality relationships of healthy, noncomplaining older men and women. J Psychosom Res 2004; 56: 503-10.

[14] Redline S, Kirchner HL, Quan SF, Gottlieb DJ, Kapur V, Newman A. The effects of age, sex, ethnicity, and sleep-disordered breathing on sleep architecture. Arch Intern Med 2004; 164: 406-18.

[15] Brendel DH, Reynolds CF3rd, Jennings JR, *et al*. Sleep stage physiology, mood, and vigilance responses to total sleep deprivation in healthy 80-year-olds and 20-year-olds. Psychophysiol 1990; 27(6): 677-85.

[16] Duffy J F, Willson HJ, Wang W, Czeisler CA. Healthy older adults better tolerate sleep deprivation than young adults. J Am Geriatr Soc 2009; 57: 1245-51.

[17] Dijk DJ, Groeger JA, Stanley N, Deacon S. Age-related reduction in daytime sleep propensity and nocturnal slow wave sleep. Sleep 2010; 33: 211-23.

[18] Landolt HP, Rétey JV, Adam M. Reduced neurobehavioral impairment from sleep deprivation in older adults: contribution of adenosinergic mechanisms. Front Neurol 2012; 3: 62.

[19] Borbély AA. A two process model of sleep regulation. Hum Neurobiol 1982; 1: 195-204.

[20] Daan S, Beersma DGM, Borbély AA. Timing of human sleep: recovery process gated by a circadian pacemaker. Am J Physiol 1984; 246: R161-78.

[21] Cajochen C, Münch M, Knoblauch V, Blatter K, Wirz-Justice A. Age-related changes in the circadian and homeostatic regulation of human sleep. Chronobiol Int 2006; 23: 461-73.

[22] Dijk DJ, Duffy JF, Riel E, Shanahan TL, Czeisler CA. Ageing and the circadian and homeostatic regulation of human sleep during forced desynchrony of rest, melatonin and temperature rhythms. J Physiol (Lond) 1999; 516(2): 611-27.

[23] Dijk DJ, Duffy JF, Czeisler CA. Age-related increase in awakenings: impaired consolidation of non REM sleep at all circadian phases. Sleep 2001; 24: 565-77.

[24] Wei HG, Riel E, Czeisler CA, Dijk DJ. Attenuated amplitude of circadian and sleep-dependent modulation of electroencephalographic sleep spindle characteristics in elderly human subjects. Neurosci Lett 1999: 260:29-32.

[25] Knoblauch V, Münch M, Blatter K, *et al*. Age-related changes in the circadian modulation of sleep spindle frequency during nap sleep. Sleep 2005; 28: 1093-01.

[26] Münch M, Knoblauch V, Blatter K, *et al*. Age-related attenuation of the evening circadian arousal signal in humans. Neurobiol Aging 2005; 26: 1307-19.

[27] Münch M, Knoblauch V, Blatter K, Wirz-Justice A, Krauchi K, Cajochen C. Homeostatic sleep regulation under low sleep pressure: are there age effects? Sleep 2007; 30(6): 781-92.

[28] Gaudreau H, Morettini J, Lavoie HB, Carrier J. Effects of a 25-h sleep deprivation on daytime sleep in the middle-aged. Neurobiol Aging 2001; 22: 461-8.

[29] Bonnet MH, Rosa RR. Sleep and performance in young adults and older normals and insomniacs during acute sleep loss and recovery. Biol Psychol 1987; 25: 153-72.

[30] Drapeau C, Carrier J. Fluctuation of waking electroencephalogram and subjective alertness during a 25-hour sleep- deprivation episode in young and middle-aged subjects. Sleep 2004; 27: 55-60.

[31] Campbell I, Feinberg I. Homeostatic sleep response to naps is similar in normal elderly and young adults. Neurobiol Aging 2005; 26: 135-44.

[32] Münch M, Knoblauch V, Blatter K, Schroder C, Schnitzler C, Krauchi K. The frontal predominance in human EEG delta activity after sleep loss decreases with age. Eur J Neurosci 2004; 20: 1402-10.

[33] Lafortune M, Gagnon JF, Latreille V, *et al*. Reduced slow-wave rebound during daytime recovery sleep in middle-aged subjects. PLoS One 2012; 7(8): e43224.

[34] Robillard R, Massicotte-Marquez J, Kawinska A, Paquet J, Frenette S, Carrier J. Topography of homeostatic sleep pressure dissipation across the night in young and middle-aged men and women. J Sleep Res 2010; 19(3): 455-65.

[35] Putilov AA, Donskaya OG, Verevkin EG, Shtark MB. Structuring the inter-individual variation in waking EEG can help to discriminate between the objective markers of sleep debt and sleep pressure. Somnologie 2009; 13: 72-88.

[36] Putilov AA. Principal components of electroencephalographic spectrum as markers of opponent processes underlying ultradian sleep cycles. Chronobiol Int 2011; 28(4): 287-99.

[37] Putilov AA. Prospects of using electroencephalographic signatures of the chronoregulatory processes for meaningful, parsimonious

and quantitative description of the sleep-wake sub-states. Biol Rhythm Res 2011; 42(3):181-207.

[38]   Putilov AA, Donskaya OG, Verevkin EG. Quantification of sleepiness through principal component analysis of the EEG spectrum**.** Chronobiol Int 2012; 29(4): 509-22.

[39]   Donskaya OG, Verevkin EG, Putilov AA. The first and second principal components of the EEG spectrum as the indicators of sleepiness. Somnologie 2012; 16(2): 69-79.

[40]   Putilov AA, Donskaya OG. Rapid changes in scores on the two largest principal components of the electroencephalographic spectrum demarcate the boundaries of drowsy sleep. Sleep Biol Rhythms 2013; 11: - (Available from: http://onlinelibrary.wiley.com/doi/10.1111/sbr.12017/abstract).

[41]   Rechtschaffen A, Kales A, eds. (1968). A manual of standardized terminology, techniques and scoring system for sleep stages of human subjects. UCLA Brain Information Service/Brain Research Institute, Los Angeles.

[42]   Silva EJ, Wang W, Ronda JM, Wyatt JK, Duffy JF. Circadian and wake- dependent influences on subjective sleepiness, cognitive throughput, and reaction time performance in older and young adults. Sleep 2010; 33: 481-90.

[43]   Henelius A, Korpela J, Huotilainen M. Individualising EEG frequency bands for sleep deprivation studies. Conf Proc IEEE Eng Med Biol Soc 2011; 2011: 6083-6.