ORIGINAL ARTICLE

Alpha attenuation soon after closing the eyes as an objective indicator of sleepiness

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SUMMARY

Attenuation of alpha rhythm in occipital derivation serves as a reliable electroencephalographic (EEG) marker of sleep onset. If such attenuation not only coincides with but also anticipates sleep onset, objective evaluation of sleepiness of permanently waking individuals might be facilitated by probing alpha attenuation immediately after closing eyes. We tested whether alpha-based EEG indexes reflect self-scored sleepiness and objectively measured waking ability. A total of 15 young adults self-scored their sleepiness before and after recording of their resting EEG with a 2-h interval in the course of 43–61-h wakefulness. For each EEG record, power spectra were calculated on 2-min intervals of the eyes open section and on five following 1-min intervals of the eyes closed section. Aking ability was assessed as latency to sleep onset marked by zero-crossing decline of such EEG indexes as alpha-theta power difference in occipital derivation and scores on the second principal component of the EEG spectrum in frontal and occipital derivations. Alpha attenuation during the first minute with eyes closed was found to be significantly related to the levels of subjective sleepiness and waking ability. The relationship between alpha attenuation and subjective sleepiness was confirmed by analysing 1-min eyes closed EEG recordings obtained with a 3-h interval in the course of 24-h sustained wakefulness of 130 adolescents and adults. We concluded that such 1-min eyes closed EEG recordings might be used for simple and quick measurements of sleepiness and waking ability in experimental and field studies of permanently waking individuals.

Key words: alertness, alpha attenuation test, electroencephalographic spectrum, Karolinska drowsiness test, sleep onset.

INTRODUCTION

Disappearance of alpha rhythm has been recognized by the founders of sleep science as a reliable electroencephalographic (EEG) indicator of transitions from wakefulness to sleep.[1] Since 1968, such diminishing of alpha activity has been used as one of the polysomnographic criteria of initiation of stage 1 sleep.[2] In the most recently published version of the standard sleep scoring rules, attenuation of alpha rhythm in occipital derivation was recommended as the best EEG marker of sleep onset.[3,4] A computerized analysis of the EEG recordings obtained in the course of wake-sleep transitions provided possibilities to relate the quantitative descriptions of alpha attenuation to certain quantitative changes in spectral composition of the EEG signal.[5–14] In particular, we recently showed that the epoch of sleep onset is usually characterized by zero-crossing decline of several alpha-based EEG indexes, such as alpha-theta power difference in occipital derivation and scores on the second principal component of the EEG spectrum in any (i.e. frontal, occipital etc.) derivation.[8–14]

However, it remains unclear whether these alpha-based EEG indexes can also respond to change in alertness–sleepiness level of still waking individuals before initiation of stage 1 sleep. The simplest objective polysomnographic tests of sleepiness, such as the alpha attenuation test[15] and the Karolinska drowsiness test,[16] combine an evaluation of attenuation of alpha rhythm in the eyes closed condition with measuring 'alpha bursts' reflecting a drowsiness-related increase of alpha power in the eyes open condition. Estimation of alpha indexes in both conditions takes time, but, anyway, the duration of such a test (i.e. between 5 and 10 min) is remarkably shorter compared with the time interval requiring for application of other methods of objective measurements of sleepiness including the Multiple Sleep Latency Test (i.e. up to 20 min).[17] However, some of the earlier reported results show that the increase of alpha power in the eyes open condition did not contribute greatly to the accuracy of distinguishing between drowsy and alert individuals as compared with the contribution of the decrease of alpha power in the eyes closed condition.[18] Therefore, the necessity of the EEG recordings in both conditions for determination of the alertness–sleepiness level can be questioned. If a testing procedure can be reduced to the EEG sampling the in eyes closed condition, this can open a perspective of remarkable simplification of a drowsiness test. For instance, its duration can be shortened to a minute or so.

In the present study, we examined whether objective evaluation of sleepiness can be facilitated by a quantitative analysis of the eyes closed EEG signal recorded at the first minute after closing the eyes. We tested whether several measures of alpha attenuation obtained during this short time interval are linked to distinct

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levels of self-reported sleepiness and objectively measured waking ability. Our hypothesis was that a statistically significant decline of the alpha-based EEG indexes not only coincides with sleep onset, but also anticipates it.

RESULTS

The preliminary analysis of log-transformed single-Hz power values provided evidence that only a few narrow (single-Hz) frequency bands showed a very close relation to subjective sleepiness measured with the KSS; and, simultaneously, these bands showed most dramatic change in the course of permanent sleep deprivation.[16] Irrespective of derivation, a highly significant time-course of spectral power values from the first to the 25th recording session ($F_{24} > 2.3$, $P < 0.001$) and very strong correlation coefficients with KSS score (<-0.9, $P < 0.001$) were shown by 10 Hz (Table 1) and 15 Hz power values in the eyes closed condition. Additionally, a highly significant time-course was also found for two adjacent powers (11 and 16 Hz in the eyes closed condition), and for the difference between 10 Hz powers in the eyes closed and open conditions. Besides, a very strong correlation was obtained for 9 Hz power in the eyes closed condition.

Therefore, the results of the preliminary analysis were interpreted as showing that spectral powers around 10 Hz frequency band exhibits the most profound changes in the course of the sleep deprivation experiment, and such changes show the strongest relationship with the changes in self-scored sleepiness. These results for 10 Hz power are shown in Table 1, and Figs 1 and 2 (lower part). Additionally, Fig. 1 (lower part) shows that 10 Hz power demonstrates rapid attenuation throughout sleep onset. Such changes in this EEG index occurred irrespective of duration of preceding wakefulness (see Methods).

As it is shown in Fig. 1, not only 10 Hz power (lower part), but also the difference between alpha and theta powers (higher part) always exhibited a rapid drop within 1-min interval of transition from wakefulness to sleep. It was noted that several alpha-based EEG indexes were different in the pattern of their change after sleep onset. Some indexes, such as scores on the second principal component in both frontal and occipital derivations, and alpha-theta difference in occipital derivation (Fig. 1), continued their decline below 0 after decline within 0 min of sleep onset. These always declining time-courses were the major reason for applying these three EEG indexes for demarcation of sleep onset event and for measurement of objective sleepiness level in minutes before such a sleep onset event marked by a zero-crossing decline of an EEG index (see Methods). In contrast, some other EEG indexes, such as alpha power and 10 Hz power (Fig. 1), appeared again after the minute of sleep onset. The pattern of change in frontal alpha-theta difference after sleep onset (Fig. 1) was found to be intermediate between these two patterns. As for the pattern of change in alpha-based EEG indexes before sleep onset, they all showed a declining trend, but this decline was less steep compared with the following decline on the boundary between wakefulness and sleep (Fig. 1).

Figures 2 and 3, and Tables 1 and 2 show the results on the evaluation of the association of the levels of objective/subjective sleepiness with the levels of alpha-based EEG indexes calculated for the first minute with eyes closed. The particular results given in Fig. 2 and Table 1 suggest a very strong link between changes in these levels in the course of experimental sleep deprivation. The eyes closed condition was characterized by the highest and highly significant coefficients of correlations between time-courses of an EEG index and objective/subjective alertness-sleepiness measures (i.e. latency to sleep onset/KSS score). The correlation coefficients were always stronger than the coefficients for preceding the open eyes condition. For instance, despite the significance of correlations obtained for the eyes open condition ($P < 0.05$), the P-values for correlation coefficients were sometimes somewhat higher than the minimal P-value of 0.001. In contrast, Table 1 shows that P-values were always below 0.001 for the eyes closed condition and for the difference between conditions.

Figure 3 and Table 2 document the major finding on the significant relationship of the alpha-based EEG indexes with four distinct levels of objective/subjective alertness–sleepiness (alertness state, and sub-states of moderate, severe and disabling sleepiness). As a rule, each of these four alertness–sleepiness levels was significantly different from the three other levels. In particular, Table 2 reports the levels of significance of such results yielded by *post hoc* pairwise comparisons. For example, two-way ANOVA of data on the eyes closed frontal alpha-theta difference detected a highly significant effect of the fixed factor 'Objective

Table 1 Correlations between time-courses of sleepiness and alpha-based electroencephalographic indexes

Sleepiness condition derivation	Objective				Subjective			
	Closed eyes		Closed – open eyes		Closed eyes		Closed – open eyes	
	Fz	Oz	Fz	Oz	Fz	Oz	Fz	Oz
2PC	−0.908***	−0.882***	−0.857***	−0.881***	−0.883***	−0.856***	−0.841***	−0.854***
ATD	−0.941***	−0.889***	−0.844***	−0.846***	−0.938***	−0.870***	−0.843***	−0.811***
Alpha	−0.930***	−0.954***	−0.933***	−0.869***	−0.937***	−0.948***	−0.900***	−0.834***
10 Hz	−0.946***	−0.962***	−0.943***	−0.878***	−0.943***	−0.946***	−0.897***	−0.845***

***$P < 0.001$ Spearman's coefficients of correlation ($n = 25$). Four different alpha-based electroencephalographic indexes were: second Principal Component score (2PC), alpha-theta power difference (ATD), alpha power (Alpha) and 10 Hz power (10 Hz). Their time-courses were correlated with the time-course of either subjective or objective sleepiness (either Karolinska Sleepiness Scale score, ranging between 1 and 9, or mean minute of eyes closed electroencephalographic recording identified as the minute of sleep onset, ranging between −5 and 0, respectively). Closed eyes or Closed – open eyes: a value of an electroencephalographic index for the first minute with eyes closed or this value minus a value calculated for the eyes open condition; Fz or Oz: Data from either frontal or occipital derivation.

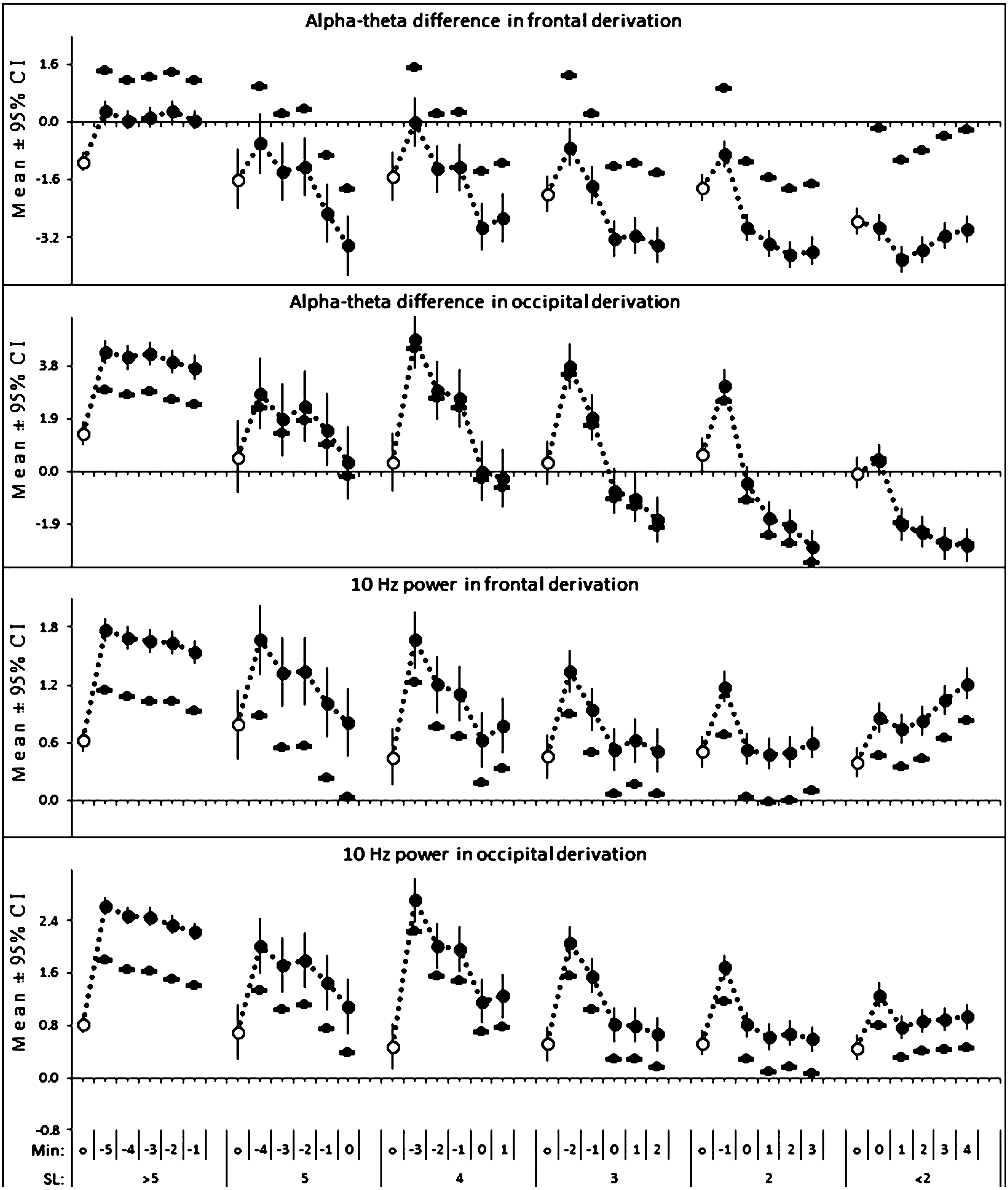

Fig. 1 Changes in alpha-based electroencephalographic (EEG) indexes before and after onset of sleep. Changes are shown on the example of two alpha-based EEG indexes, alpha-theta power difference (upper plots) and 10 Hz power (lower plots). Sleep onset (Min 0) was determined by applying the criterion of downward zero-crossing of the second principal component score in frontal derivation. Six values of an EEG index were assigned relative to sleep onset, sleep onset latencies (SL) varied from > 5 (wakefulness without any signs of sleep) to < 2 (sleep onset already within the first min with eyes closed). Group-averaged values of the EEG indexes (dotted lines connecting open and closed circles for eyes open and closed condition, respectively) were obtained by within- and then across-subject averaging, and shown with 95% confidence interval (CI) of the mean (vertical bars, but results of statistical analysis are not reported). Additionally, group-averaged differential EEG indexes were computed as differences between a value obtained for one of five consecutive 1-min eyes closed intervals and a value for the preceding eyes open (o) condition (closed circles crossed by dashes).

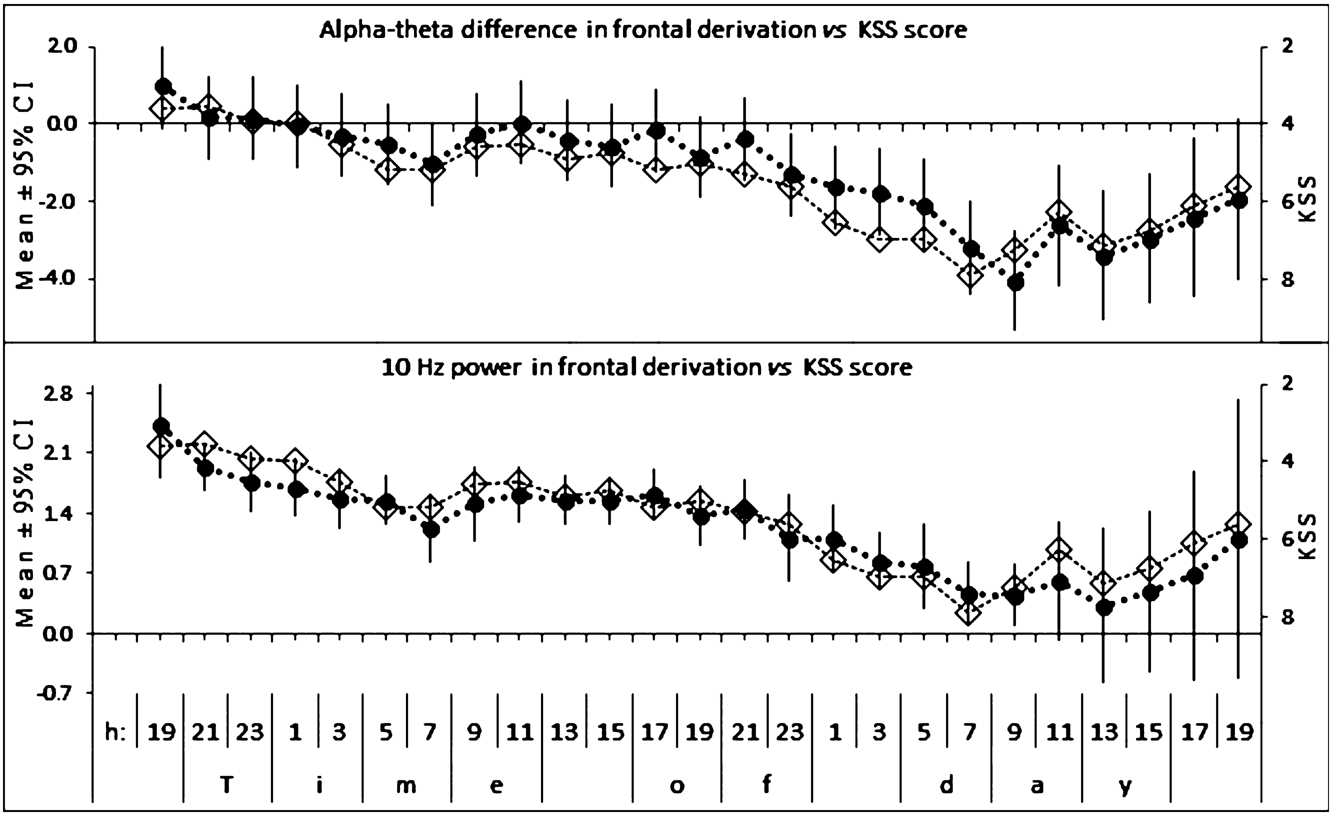

Fig. 2 Correlations between time-courses of alpha-based electroencephalographic (EEG) indexes and subjective sleepiness. Time-courses of frontal alpha-theta difference (upper part) and frontal 10 Hz power (lower part) were obtained for the first 1-min interval of the eyes closed section of the EEG records (closed circles with dotted lines). Averaged values of EEG indexes are shown with ± 95% confidence interval (CI) of the mean (vertical bars).

sleepiness' $F(n = 15, \text{d.f.} = 3) = 28.4$ ($P < 0.001$) with a significant pairwise difference between any pair of sleepiness levels (Table 2). The effect of the fixed factor 'Subjective sleepiness' was also highly significant, $F(n = 15, \text{d.f.} = 3) = 11.8$ ($P < 0.001$), and, again, all pairwise differences were found to be significant (Table 2). These ANOVA results were supported by the results of one-way RANOVA ('Objective sleepiness'/'Subjective sleepiness' as within-subjects repeated measure). For example, RANOVA of data on the eyes closed frontal alpha-theta difference in participants showing all four levels of objective/subjective alertness–sleepiness yielded highly a significant effect of sleepiness level, $F(n = 9, \text{d.f.} = 3) = 34.7$ ($P < 0.001$)/$F(n = 7, \text{d.f.} = 3) = 15.2$ ($P < 0.001$), with results of *post hoc* pairwise comparisons suggesting that, in particular, the highest sleepiness level significantly differed from any of the three other levels (-2.39, $P < 0.0$/-1.86, $P < 0.01$, -4.25/$P < 0.01$, -3.06, $P < 0.05$ and -4.59, $P < 0.001$/-4.22, $P < 0.05$).

Overall, the closest links with sleepiness were shown by the alpha-based EEG indexes calculated for the first minute with eyes closed as compared with the preceding eyes open condition and the difference between conditions. Furthermore, these links were stronger when sleepiness was measured objectively (as sleep onset latency). For instance, the difference between two adjacent levels corresponding to severe and disabling sleepiness was not sometimes statistically significant for subjectively assessed sleepiness, whereas this difference was always highly significant for objective measure of sleepiness (Table 2). This was not an unexpected result, because such a disabling level of objective sleepiness signifies sleep onset already after closing the eyes, whereas extremely drowsy individuals often tend to underestimate the severity of the deterioration of their alertness and performance.

Table 3 and Fig. 4 (left plots) show the results supporting the aforementioned results on the link between attenuation of an alpha-based EEG index and subjective sleepiness. These results were obtained in analysis of the independent dataset including 1-min eyes closed sections of the EEG recordings from 130 healthy participants. They suggest that the similarity between the time-courses of the alpha-based indexes and KSS score was remarkable even during the first day of sustained wakefulness (Fig. 4, right plots). Furthermore, two-way ANOVA (Table 3) detected a highly significant main effect of the fixed factor 'Subjective sleepiness'. For instance, the ANOVA of data on frontal alpha-theta difference gave $F(n = 130, \text{d.f.} = 3) = 24.7$ ($P < 0.001$) with significant pairwise differences between any of the six pairs of subjective sleepiness levels. These findings were supported by the results of one-way RANOVA. For instance, such RANOVA of eyes closed frontal alpha-theta difference yielded a highly significant effect of within-subjects repeated measure 'Subjective sleepiness', $F(n = 31, \text{d.f.} = 3) = 13.6$ ($P < 0.001$), with *post hoc* pairwise comparisons suggesting a significant difference of the highest subjective sleepiness level from any of the three other alertness–sleepiness levels (-0.27, $P < 0.05$, -0.37, $P < 0.01$ and -0.42, $P < 0.001$). Overall, such results of experimental 24-h sleep deprivation confirmed the usefulness of the alpha-based EEG indexes for discrimination between distinct subjective sleepiness levels (Fig. 4).

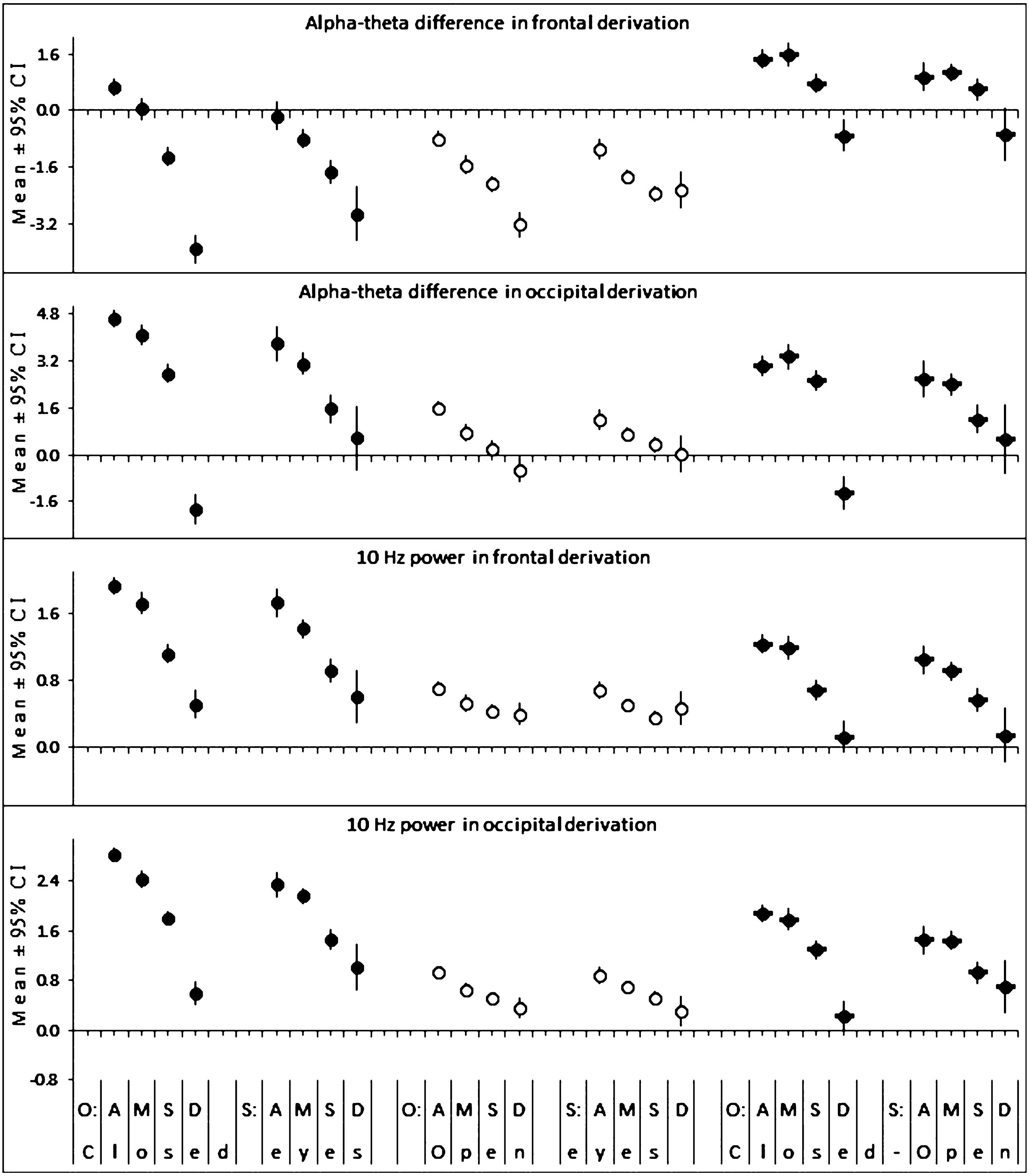

Fig. 3 Alpha-based electroencephalographic (EEG) indexes for four distinct levels of objective and subjective sleepiness. Alpha-theta power difference and 10 Hz power (upper and lower plots, respectively) were calculated for the first 1-min eyes closed interval, eyes open condition and the difference between them (closed circles, open circles, closed circles crossed by dashes, respectively). Group-averaged EEG indexes with ± 95% confidence interval (CI) of the mean (vertical bars) were obtained through within- and then across-individual averaging of values corresponding to four distinct sleepiness levels: Alert (A) state or minimal sleepiness, mild or Moderate (M), marked or Severe (S) and Disabling (D) sleepiness. O, objective alertness–sleepiness determined as the mean minute of sleep onset. S, subjective alertness–sleepiness determined from the Karolinska Sleepiness Scale score.

DISCUSSION

Attenuation of alpha rhythm in occipital derivation was recommended as the best EEG marker of sleep onset,[2,3] and zero-crossing declines of some of alpha-based spectral EEG indexes can serve as simple and reliable yes-or-no criteria for identification of sleep onset in different cortical areas.[9–13] If alpha rhythm starts

Table 2 Differences between the electroencephalographic indexes for four levels of objective and subjective sleepiness

Index	Alpha-theta power difference				10 Hz power			
Objective	Disabling	−4.6***	−5.9***	−6.5***	Disabling	−1.2***	−1.8***	−2.2***
	−2.6***	Severe	−1.3***	−1.9***	−0.6***	Severe	−0.6***	−1.0***
	−4.0***	−1.4***	Moderate	−0.5	−1.2***	−0.6***	Moderate	−0.4***
	−4.6***	−2.0***	−0.6*	Alert	−1.4***	−0.8***	−0.2	Alert
Subjective	Disabling	−1.0	−2.5***	−3.2***	Disabling	−0.4	−1.1***	−1.3***
	−1.2*	Severe	−1.5***	−2.2***	−0.3	Severe	−0.7***	−0.9***
	−2.1***	−0.9***	Moderate	−0.7	−0.8***	−0.5***	Moderate	−0.2
	−2.8***	−1.6***	−0.7*	Alert	−1.1***	−0.8***	−0.3*	Alert

***$P < 0.001$, **$P < 0.01$, *$P < 0.05$: the level of significance of t-value in *post hoc* pairwise comparisons of an electroencephalographic index for the four levels of a sleepiness factor (with Bonferroni adjustment for the number of comparisons). Differences between values of the alpha-based electroencephalographic indexes calculated for the first minute with eyes closed (frontal and occipital derivations are shown below and above the diagonal, respectively). Results were obtained by applying two-way ANOVA with a fixed factor 'Objective/Subjective sleepiness' (4 levels: Disabling, Severe or marked, Moderate or mild, and minimal sleepiness or Alert state) and a random factor 'Participant' (n = 15).

Table 3 Differences between the electroencephalographic indexes calculated for four levels of subjective sleepiness

Index	Alpha-theta power difference				10 Hz power			
Subjective	Disabling	−0.3***	−0.4***	−0.5***	Disabling	−0.3**	−0.4***	−0.5***
	−0.2***	Severe	−0.1	−0.2***	−0.1	Severe	−0.2***	−0.2***
	−0.3***	−0.1*	Moderate	−0.1	−0.3***	−0.2***	Moderate	−0.0
	−0.4***	−0.2***	−0.1**	Alert	−0.3***	−0.2***	−0.0	Alert

***$P < 0.001$, **$P < 0.01$, *$P < 0.05$: the level of signicance of t-value in *post hoc* pairwise comparisons of an electroencephalographic index for the four levels of a sleepiness factor (with Bonferroni adjustment for the number of comparisons). Difference between relative values of the alpha-based electroencephalographic indexes calculated for one-min interval of eyes closed section of the electroencephalographic records from frontal and occipital derivations (below and above the diagonal, respectively). Results were obtained by applying two-way ANOVA with a fixed factor 'Subjective sleepiness' (4 levels) and a random factor 'Participant' (n = 130).

its significant attenuation already before sleep onset, objective detection of reduced alertness level might be facilitated by measuring the attenuation of the alpha-based EEG indexes right after closing the eyes. We applied the quantitative EEG analysis to data obtained from sleep-deprived individuals for testing the suggestion that a quick estimation of levels of these alpha-based EEG indexes in eyes closed condition can provide a simple, but reliable, and practically useful measure of increased sleepiness and decreased waking ability. We found that attenuation of such indexes was significantly associated with an elevated feeling of sleepiness and shortened wakefulness interval before sleep onset as a result of the decline of waking ability. The first of these two associations was supported by the results of the analysis of an additional dataset.

Experimental research of regulatory processes underlying the sleep–wake cycle can benefit from implementation of such 1-min eyes closed EEG measurements in studies of permanently waking individuals. Circadian and sleep researchers might prefer to collect the EEG data in the eyes open rather than eyes closed condition during their sleep deprivation experiments, because they reasonably suspect that study participants will very rapidly fall asleep after closing their eyes to 'pay' back some amount of accumulated sleep debt. Indeed, slow-wave activity shows a rapid rise of right after sleep onset. However, we earlier showed that: (i) the slow-wave activity index can be viewed as representing the difference between scores on the first and second principal components of the EEG spectrum; (ii) 'payment' of sleep debt can be associated only with a rise of the first principal component score; and (iii) this rise is always after the interval of transition to sleep associated with a rapid decline of the second principal component score.[9–11,20] Therefore, if sleepiness was measured only during the 1-min interval after closing the eyes, such a recording session was terminated before the initiation of the rapid rise of the first principal component score. Consequently, multiple quick measurements of objective sleepiness level can be carried out throughout the whole sleep deprivation experiment without interrupting the phase of gradual accumulation of sleep debt by the short phases of its 'payment'.

The present results also have practical relevance to the development of user-friendly instruments for field studies of sleepiness and waking ability. On the one hand, self-scoring of sleepiness on such scales as the KSS are regarded as the quickest and simplest way of testing the levels of drowsiness of sleep-deprived individuals in real-life situations, and the implementation of this reasonably accurate and valid instrument of sleepiness assessment in field studies does not require any equipment.[21] On the other hand, experimental observations also suggest that self-perceived levels of sleepiness did not always correspond reliably to the objectively measured levels.[22–25] For example, similar to the effects of alcohol, the negative effects of drowsiness are always subjectively

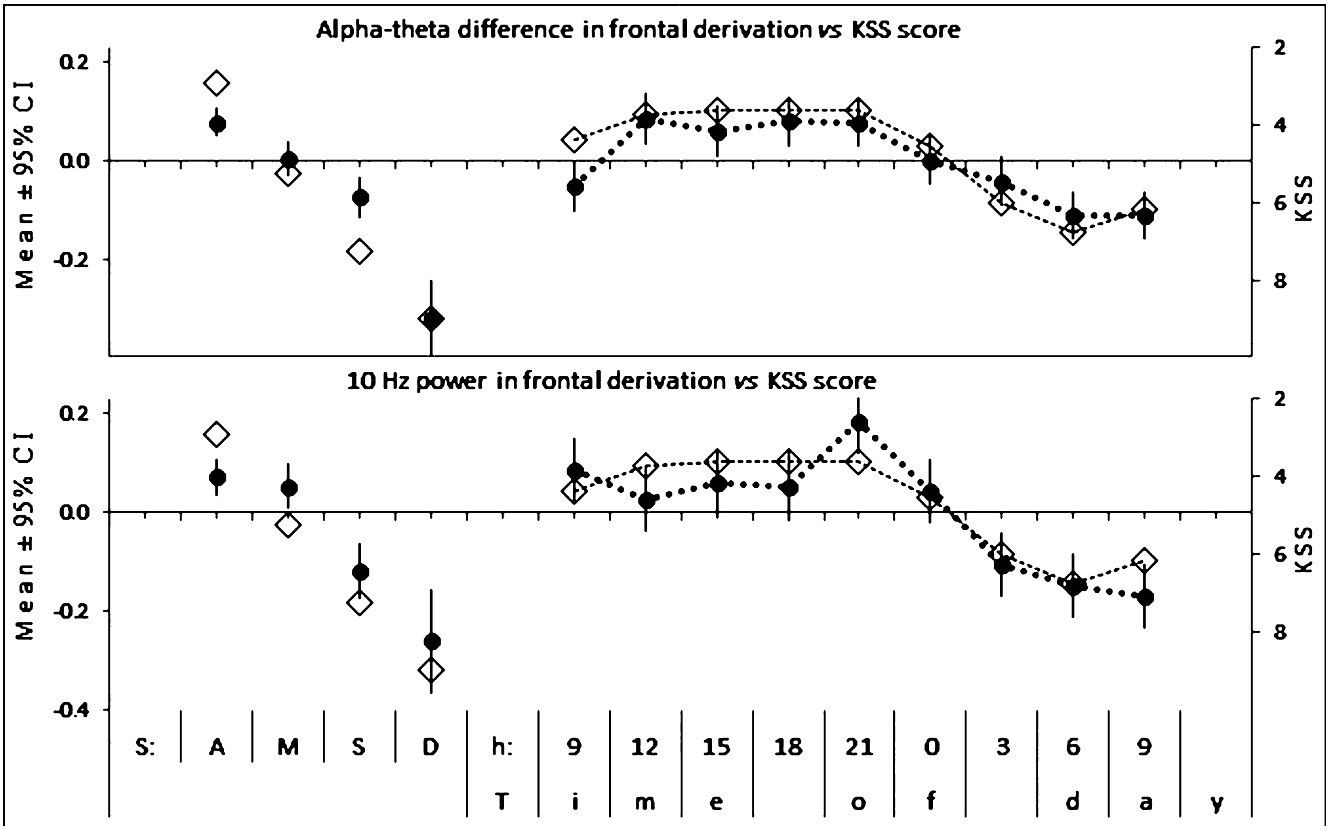

Fig. 4 Alpha-based electroencephalographic (EEG) indexes for four distinct levels of subjective sleepiness (left) and the correlation between time-courses of alpha-based EEG indexes and subjective sleepiness (right). Alpha-based EEG indexes were frontal difference between alpha and theta powers (upper plots) and frontal 10 Hz power (lower plots) obtained on 1-min interval with eyes closed. Values of an EEG index with ± 95% confidence interval (CI) for four distinct sleepiness levels (closed circles) were obtained by means of within- and then across-individual averaging of values corresponding to four distinct sleepiness levels: Alert (A) state or minimal sleepiness, mild or Moderate (M), marked or Severe (S) and Disabling (D) sleepiness. Time-courses consisting of nine time-points (right plots) were obtained by averaging across participants. Spearman's coefficients of correlation of these time courses with the corresponding time course of Karolinska Sleepiness Scale (KSS) scores (open diamonds) attained the values of −0.800 and −0.850 (upper and lower right plots, respectively, $P < 0.01$ for both).

underestimated.[26] The major reason for this might be the inability of a sleepy individual to accurately identify a level of his/her drowsiness because of impaired cognitive functioning.[27,28] Therefore, the lack of practical tools for objective measurement of sleepiness has become one of the critical barriers to reducing the threats of increased sleepiness to public health, productivity and safety.[26,29,30] The present study results show that the EEG recordings in both eyes open and closed conditions might not always be necessary for accurate evaluation of changes in alertness-sleepiness level.[15,16] Therefore, much shorter (e.g. approximately 1 min instead of 5–10 min) recording in the eyes closed condition appears to be sufficient for detecting these changes.

Further studies might aim to confirm the possibility of using such 1-min closed eyes recordings for a comparatively simple, quick, cheap and minimally-intrusive, but still objective, measurement of waking ability and physiological sleepiness in sleep-deprived individuals. As the present study results are based on group-averaged data, they do not take into account the earlier reported cases of remarkable individual differences in the characteristics of alpha rhythm[31] and in the relationship between these characteristics and alertness–sleepiness levels.[16,21] Therefore, future research might also aim to detect possible difficulties for the application of 1-min closed eyes recordings for evaluating sleepiness in individuals without prominent alpha activity, unusually fast alpha rhythm (< 9 Hz) and so on.

To conclude, the present analysis of the first 1-min interval of the EEG signal recorded immediately after closing the eyes (i.e. before sleep onset) showed that different alpha attenuation indexes can reflect decreased levels of alertness and waking ability. Implementation of such analysis in experimental and field studies can facilitate objective measurement of sleepiness caused by sleep deprivation and restriction.

METHODS

The analysed databases consist of 315 and 1170 sets of resting EEG recordings collected during experimental sleep deprivation of 15 young adults, and 130 adolescents and adults, respectively. Both studies were carried out in accordance with the ethical standards laid down in the Declaration of Helsinki. Their protocols were approved by the ethics committee of the Siberian Branch of the Russian Academy of Medical Sciences. Informed written consent was obtained from each of the participants studied as paid volunteers.

Participants of the first study were practically healthy young males ($n = 7$) and females ($n = 8$) with ages ranging from 22 to

26 years and from 19 to 24 years (mean ± SD: 24.7 ± 1.9 and 21.6 ± 2.2, respectively). The EEG signal was recorded with a 2-h interval in the eyes open and eyes closed conditions (2 and 5 min, respectively) starting from Friday evening (19.00 hours). The last session was scheduled on Sunday evening (19.00 hours). Each participant could terminate his/her participation in the experiment when they felt the irresistible desire to sleep during one of the 2-h intervals between the EEG recordings. All participants completed at least 16 recording sessions, but just four of them completed all 25 sessions. Before and after each EEG recording, the participants self-rated their alertness–sleepiness on the nine-point the Karolinska Sleepiness Scale (KSS); that is, the nine-point verbally anchored scale with the following steps: 1 = very alert; 3 = alert; 5 = neither alert nor sleepy; 7 = sleepy, but no problem staying awake; and 9 = very sleepy, great effort to keep awake.[16] The intermediate steps are not anchored verbally. The mean KSS scores obtained by averaging the self-reported scores before and after each EEG recording were used in the present analysis as indicators of subjective sleepiness.

Two (frontal and occipital) electrodes were used for the EEG recordings. The first active electrode was placed in the middle from the top of the head to forehead (Fz–A2), and the second was placed midway between O1 and O2 of the international 10–20 electrode system (Oz–A2). To fix the electrodes, Ten 20 conductive paste was used (Nicolet Biomedical, Madison, WI, USA). The exact positions of the active electrodes were preliminary inked by permanent marker, and the electrodes were removed after each recording session. The EEG signals were recorded by a 16-channel electroencephalograph (Neuron-Spectrum-2, Neurosoft, Ivanovo, Russia), conditioned by the high-pass, low-pass and notch filters (0.5, 35 and 50 Hz, respectively), sampled and stored on a hard disc with a frequency of 200 Hz. The EEG signals were inspected visually, and 2-s epochs containing artifacts were removed from further analysis. Power spectra for artifact-free 2-s epochs were computed using the FFTW (the Fastest Fourier Transform in the West) package (Free Software Foundation, Boston, MA, USA).[19] Absolute spectral powers (μV^2) were calculated for single-Hz frequency bandwidths (i.e. 0.50–1.49, 1.50–2.49, … 31.50–32.49 Hz). The spectra of the artifact-free 2-s epochs were averaged within each of the six intervals of the EEG record; that is, on one 1-min eyes open section and on five following 1-min intervals of the eyes closed section.

The absolute power values in the frequency range from 1–16 Hz were converted into a natural logarithmic scale. All statistical analyses were carried out with the SPSS statistical software package (version 20.0; IBM, Armonk, NY, USA). The preliminary analysis of data for each of these log-transformed single-Hz powers was carried out for determining which frequency is most responsible for the sleep deprivation procedure and most closely associated with subjective sleepiness levels. Three time-courses of a single-Hz power were calculated for the eyes open condition, the following 1-min interval of the eyes closed condition and the difference between them. The significance of the main effect of the factor; Time' (25 time-points) was tested with one-way ANOVA. The association between such a time-course and the time-course of KSS score was evaluated with Spearman's coefficients of correlation.

The 16 log-transformed single-Hz power values were also used for calculation of alpha-based EEG indexes, such as power in alpha range (9–12 Hz), difference between powers in alpha and theta (5–8 Hz) ranges, and scores on the second principal component of the EEG spectrum (the details on the calculation of these scores were provided in our earlier publications).[8,9,14] Similarly to the time-courses of single-Hz powers, the time-courses of these alpha-based EEG indexes were correlated with the time-course of KSS score.

In order to show changes in the EEG indexes around sleep onset, the 7-min EEG recording sessions were sorted into six groups in accord with the duration of wakefulness; that is, from only wakefulness to sleep initiated during the first minute with eyes closed. A minute of sleep onset was determined as a minute during which an EEG index changed its level from the last positive to the first negative value.[10–13] The mean minute of sleep onset was obtained by averaging over sleep onset minutes identified by applying zero-crossing criteria to within-session time-courses of such three EEG indexes as occipital alpha-theta difference, and frontal and occipital scores on the second principal component of the EEG spectrum. This mean value was then used in analysis aimed at establishing a relationship between an alpha-based EEG index and objective sleepiness level. In this analysis, four distinct levels of waking ability were distinguished: alert state (only wakefulness), and moderate, severe and disabling sleepiness substates (more or < 2.5 min of wakefulness with eyes closed, and wakefulness with eyes open only; n = 105, 60, 104 and 46, respectively). Four similar levels of subjective sleepiness were established using KSS score (4 or lower, between 4.5 and 6, between 6.5 and 8, and higher than 8; n = 84, 135, 83 and 13, respectively).

The significance of differences between these four levels of alertness–sleepiness on the EEG indexes was tested by applying two-way ANOVA with a fixed factor 'Objective/Subjective sleepiness', and a random factor 'Participant' (n = 15). The Bonferroni multiple comparison test was used in the *post hoc* analysis to examine the significance of pairwise differences between alertness–sleepiness levels. To confirm the results of these ANOVA, one-way repeated measure ANOVA were carried out after within-individual averaging of values of an EEG index corresponding to four distinct levels of objective/subjective sleepiness. The Huynh–Feldt correction of the degrees of freedom was used to control for type 1 error associated with violation of the sphericity assumption. As some participants were characterized by less than four distinct levels of sleepiness, these rANOVA and *post hoc* pairwise comparisons were based on subsamples of nine and seven study participants ('Objective sleepiness' and 'Subjective sleepiness', respectively).

Participants of the second study were practically healthy adolescents and adults. The mean ages of 54 male and 76 female participants were 27.4 ± 10.1 years (range from 15 to 55 years) and 30.8 ± 13.4 (range from 16 to 66 years), respectively. In the experimental morning (between 08.00 and 8.30 h), they were admitted to a research unit of the institute, and remained there until approximately 11.00 hours the next morning. Over the next 24 h, each study participant completed nine 3–4-min EEG recording sessions divided by 3-h intervals. KSS was self-scored after each session. The EEG recordings were taken at frontal and occipital scalp sites corresponding to derivations Fz and O2 referenced to the right mastoid, A2, of the international 10–20 electrode system. For one half of the participants, the recordings with closed eyes were obtained before the recordings with eyes open; and for another half of the participants, the eyes closed recordings followed the eyes open recordings.

In the present analysis, only data on at least 1 min of the eyes closed section of each EEG recording were used to calculate the same alpha-based EEG indexes as were obtained from the first study dataset. In order to minimize the influence of age differences on these indexes (i.e. such as age-related changes in the amplitude of alpha rhythm), the log-transformed values were expressed as deviations from the individual mean value obtained by averaging over nine measurements (from 09.00 to 09.00 hours the next day). Changes in these EEG indexes from the first to the ninth recording session were correlated with the corresponding changes in KSS score. Significance of differences between EEG indexes calculated for four distinct levels of subjective sleepiness was tested using two-way ANOVA with 'Subjective sleepiness' (four levels) as a fixed factor, and 'Participant' (n = 130) as a random factor. The ANOVA were followed by the *post hoc* analysis of pairwise differences between these four levels of subjective sleepiness. The results of ANOVA were confirmed by applying rANOVA to data from those participants who self-reported four distinct levels of sleepiness (n = 31).

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