Two circadian rhythms in the human electroencephalogram during wakefulness

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Aeschbach, Daniel, Jeffery R. Matthews, Teodor T. Postolache, Michael A. J. Jackson, Holly A. Giesen, and Thomas A. Wehr. Two circadian rhythms in the human electroencephalogram during wakefulness. Am. J. Physiol. 277 (Regulatory Integrative Comp. Physiol. 46): R1771–R1779, 1999.—The influence of the circadian pacemaker and of the duration of time awake on the electroencephalogram (EEG) was investigated in 19 humans during ~40 h of sustained wakefulness. Two circadian rhythms in spectral power density were elucidated. The first rhythm was centered in the theta band (4.25–8.0 Hz) and exhibited a minimum close to the body temperature minimum. The latter rhythm showed a close temporal association with the circadian rhythm in sleep propensity and thus coincided with the phase at which the circadian rhythm in sleep propensity is expected to be at its minimum. The nadir in a 9.25- to 12.0-Hz band coincided with the temperature minimum, a phase at which waking EEG power is typically high and alertness is low. Differences in the circadian timing suggest different functional significance of the corresponding EEG activities. However, our previous study had limitations in that it was based on broad frequency bands, which introduce nonphysiological discontinuities and result in loss of information. In the present study, we used narrow (1 Hz) frequency bands, which previously allowed researchers to identify distinctly regulated frequency components in both the sleep EEG (2) and waking EEG (21). To ensure adequate statistical power, we expanded the subject population of our preliminary study. We hypothesized that during sustained wakefulness, the EEG undergoes frequency-specific changes that can be attributed to differences in the strength of circadian and homeostatic influences as well as to differences in the timing of the circadian modulation.

The circadian rhythm of plasma melatonin appears to be critically involved in the circadian regulation of sleep propensity (35, 44). If there is an electrophysiological correlate of sleep propensity during wakefulness, a temporal association between the endogenous melatonin rhythm and the circadian modulation of the EEG is expected. To clarify this point, we recorded the EEG and measured plasma melatonin levels at regular intervals around the clock in a subgroup of subjects.

METHODS

Subjects. Nineteen subjects (11 men, 8 women; age 21–31 yr) who participated in a study of the regulation of habitual sleep duration contributed to the database. Subjects were in good health, as assessed by medical history, structured clinical interview, physical exam, electrocardiography, and biochemical screening. They reported no sleep problems or shift work and no use of medications, drugs, or tobacco. Subjects had a stable sleep-wake pattern as assessed by questionnaires, 2- to 4-wk sleep logs, and wrist motor activity recorders.
nings before the study. Six individuals habitually slept 6 h or less per night, eight individuals slept 7–8.25 h, and five individuals slept 9 h or more. Women were studied during the follicular phase of their menstrual cycle, as determined by logs and ovulation kits (Clearplan Easy, Unipath, Bedford, UK). For 1 wk before the study, subjects were instructed to refrain from alcohol and caffeinated products and to maintain their habitual bedtimes and wake-up times. Wrist-worn activity monitors were used to check compliance with the latter instruction.

Protocol. The study protocol was approved by the Institutional Review Board of the National Institute of Mental Health. Subjects gave written informed consent before participating. They were admitted to a research ward for two nights of sleep, a CR protocol, and a period of recovery sleep. During the first two nights, time in bed was scheduled to correspond with each individual’s habitual bedtime (mean ± SE: 2335 ± 19 min, n = 19) and wake-up time (0703 ± 20 min). The CR began at wake-up time after the second night’s sleep and ended at 2300 the following day. The duration ranged between 37 and 42 h. During the CR, subjects stayed awake in bed in a propped-up position (upper part of bed at 45°) in a sound-attenuated room. Light intensity was <10 lx at eye level. Subjects had no access to clocks, and the staff members attending the subjects were trained not to provide any time cues. Fluids and isocaloric meals were given at room temperature every hour and every 2 h, respectively. Every half hour, the subjects rated their alertness and mood on bipolar 100-mm visual analog scales. The ratings were followed by a 3-min recording of the EEG, electrooculogram (EOG), and electromyogram (EMG). Subjects had been instructed to relax during the recordings, keep their eyes open, and focus on a spot on the wall while avoiding frequent eye blinks and movements. Acoustic signals generated on a computer indicated the time for the ratings as well as the beginning and end of the recordings. A staff member attended the subjects throughout the CR to prevent them from falling asleep and to ensure adherence to the protocol.

Polycyclic recordings. A total of 1,498 3-min recordings of the EEG (C3/A2 and C4/A1), EOG, and submental EMG was collected. The signals were amplified (Grass 7P511I), time constants for EEG and EOG: 0.9 s, EMG: 0.03 s), low-pass filtered (Cauer filter, –0.1 dB at 34 Hz, 80 dB/octave), digitized (sampling rate: 128 Hz, resolution: 12 bit), and stored on magneto-optical disk. The EEGSYS software (Friends Medical Science Research Center, Baltimore, MD) was used for data acquisition and display of the signals on a personal computer. Throughout the CR, the quality of the recordings was monitored by a trained staff member. The electrode impedance was checked at intervals of 6–10 h. All recordings were visually inspected by the same scorer, and 4-s epochs contaminated by eye blinks, eye movements, body movements, or sleep stage 1 were excluded from further analysis (i.e., mean ± SE: 47.3 ± 3.1% of recording time, n = 19). The EEGs were subjected to a fast-Fourier transform analysis (i.e., mean

The circadian component was examined in each subject and 1-Hz bin were expressed as a percentage of the mean value in that subject and bin during the CR. A value representing time relative to the fitted temperature minimum was assigned to each EEG sample. To estimate circadian and wake-dependent components in the EEG, a function with a 24-h cosine component and a saturating exponential component was fitted to power densities in each 1-Hz bin in the range of 0.25–20.0 Hz

\[ P(t) = A \cos \left( \frac{2\pi}{24} (t - t_{\text{max}}) \right) + P_{\text{c}} - (P_{\text{c}} - P_{0}) e^{-(t-t_\text{i})/\tau} \]

In this function, \( P(t) \) represents power density at time \( t \) relative to the temperature minimum, \( A \) is the amplitude of the cosine component, \( t_{\text{max}} \) is the time of its maximum if \( A > 0 \), \( P_{\text{c}} \) is the value of power density if \( t \) approaches \( \infty \) and if \( A = 0 \), \( P_{0} \) is the value of power density at wake-up time \( t_{0} \) if \( A = 0 \), and \( \tau \) is the time constant of the exponential component. The function was fitted with a nonlinear least squares regression procedure (SAS Institute). No boundaries were set for the calculation of the parameters \( A \), \( t_{\text{max}} \), \( P_{\text{c}} \), \( P_{0} \), and \( \tau \). The data entered in the regression analysis were pooled individual means over three consecutive half-hourly values.

Statistics. The circadian component was examined in each 1-Hz bin by the following method. First, the residuals of the wake-dependent component, which was given by the parameter estimates \( P_{\text{c}} \), \( P_{0} \), and \( \tau \), were expressed as a function of time relative to the temperature minimum. The fit of the cosine component through the residuals was then compared with the fit of a zero-amplitude model by using an F test criterion on the residual variances (38). If the cosine model
provided a better fit ($P < 0.05$), the residuals of the wake-dependent component were subjected to a one-way ANOVA [factor 1.5-h interval relative to temperature minimum, degrees of freedom (df) = 29]. The educed wake-dependent component was examined accordingly: the residuals of the circadian component, which was given by the parameter estimates $A$ and $t_{\text{max}}$, were expressed as a function of time awake. If the saturating exponential model provided a better fit than a horizontal line (F test), the residuals of the circadian component were subjected to a one-way ANOVA (factor 1.5-h interval since wake up, df = 28). The results of the ANOVAs are reported in Fig. 3.

**RESULTS**

Circadian and wake-dependent components in EEG power density. The EEG exhibited prominent changes during $\sim 40$ h of sustained wakefulness (Fig. 1). Two types of changes in power density were evident: non-monotonic changes that were associated with the changes in circadian phase and a global increasing trend that was associated with the increasing time awake. The temporal changes varied across EEG frequency bins. This variation was attributed to differences in the strength of circadian and wake-dependent influences. Estimation of the two influences by the simultaneous fit of a 24-h cosine component and a saturating exponential component to power densities (see METHODS) is exemplified for three 1-Hz bins in the theta, alpha, and beta bands (Fig. 2). In the 5.25- to 6.0-Hz bin, strong circadian and wake-dependent components were evident. In the 10.25- to 11.0-Hz bin, only the circadian component was statistically significant, whereas in the 17.25- to 18.0-Hz bin, only the wake-dependent component reached significance. In Fig. 3, the strength of the two components was compared for each 1-Hz bin in the range of 0.25–20.0 Hz. The plotted values represent the changes in power density during a 16-h waking episode, as estimated on the basis of the two educed components. For the frequencies $\leq 9$ Hz, prominent circadian and wake-dependent changes were obtained, the magnitude of the latter being consistently larger than the former. In the 10.25- to 12.0-Hz range, significant changes were attributed only to the circadian component. At higher frequencies, wake-dependent changes were found above 12 Hz, whereas the circadian component vanished and no longer reached significance above 14 Hz.

The kinetics of the educed wake-dependent component of power density in the beta band (13.25–20.0 Hz) differed from those in the theta (4.25–8.0 Hz) and delta (0.75–4.0 Hz) bands. This was evident from the estimates of the time constant [$\beta$: $\tau = 8.8$ h (4.1–13.4 h, asymptotic 95% confidence interval); theta: $\tau = 23.9$ (14.4–33.3) h; delta: $\tau = 16.6$ (9.9–23.2) h] and the asymptotic power density value [$\beta$: $P_\infty = 103.4$ (100.1–106.8)%; theta: $P_\infty = 136.0$ (121.0–151.0)%; delta: $P_\infty = 116.1$ (109.2–123.1)%].

Timing of the circadian variation in EEG power density. The timing of the circadian modulation of power density varied with EEG frequency. This is illustrated in Fig. 4, where the residuals of the educed wake-dependent component were color coded and expressed as a function of RCT and frequency. Two
distinct frequency bands with large circadian modulation and differences in the phase relationship to the circadian rhythm of body temperature were evident: the theta band and the high-frequency alpha band (10.25–13.0 Hz). The circadian trough in the latter occurred close to the temperature minimum and several hours after the trough in the theta band (see the 2 dark blue areas). This bimodal pattern was also evident from the estimates of amplitude and nadir time of the reduced circadian components (Fig. 5). The nadir occurred 5.73 and 0.76 h before the temperature minimum in the 5.25- to 6.0-Hz and 10.25- to 11.0-Hz bin, respectively. Comparisons of 1-Hz bins in the 0.25- to 9.0-Hz range with 1-Hz bins in the 10.25- to 13.0-Hz range revealed no or little overlap of the nadir time's asymptotic 95% confidence intervals.

Two circadian rhythms of power density, with distinct phase angles and maximal amplitudes in the theta band and high-frequency alpha band, were evident: the theta band and the high-frequency alpha band (10.25–13.0 Hz). The circadian trough in the latter occurred close to the temperature minimum and several hours after the trough in the theta band (see the 2 dark blue areas). This bimodal pattern was also evident from the estimates of amplitude and nadir time of the reduced circadian components (Fig. 5). The nadir occurred 5.73 and 0.76 h before the temperature minimum in the 5.25- to 6.0-Hz and 10.25- to 11.0-Hz bin, respectively. Comparisons of 1-Hz bins in the 0.25- to 9.0-Hz range with 1-Hz bins in the 10.25- to 13.0-Hz range revealed no or little overlap of the nadir time's asymptotic 95% confidence intervals.

The minimum of the circadian variation in theta activity (power density in the 4.25- to 8.0-Hz band), as determined in each individual by applying a three-point moving average to the residuals of the wake-dependent component, occurred 1.0 ± 0.5 h (mean ± SE, n = 10; P < 0.09, t-test for difference from 0) after the onset of melatonin secretion (Fig. 6). When referenced to the onset of melatonin secretion, the circadian trough in theta activity in the present study and the trough in sleep propensity in a multiple nap study coincided [Fig. 6, reanalysis of data from Wehr (43)].

The circadian variation in high-frequency alpha activity (power density in the 10.25- to 13.0-Hz band) was similar to the circadian variation in subjective alertness and showed a close association with the circadian rhythms of plasma melatonin and body temperature (Figs. 7 and 8). Alertness correlated positively with EEG power density in the high alpha range and negatively with power density in some 1-Hz bins in the theta and delta bands (Fig. 8). The positive correlations were attributed to the similarities in the circadian timing of the two variables. The negative correlations were attributed to the wake-dependent changes in alertness and EEG power density in the theta and delta bands, which were opposite for the two variables.

**DISCUSSION**

During sustained wakefulness, the human EEG undergoes pronounced changes. The present analysis shows that these changes can be attributed in part to circadian and wake-dependent processes. The data substantiate and extend recent findings of manifestations in the waking EEG of these two sleep-regulatory processes (4, 14). In particular, it is shown that the strength of circadian and wake-dependent influences as well as the timing of the circadian modulation of the waking EEG varies as a function of EEG frequency.

Two circadian rhythms of power density, with distinct phase angles and maximal amplitudes in the theta band and high-frequency alpha band, were evi-
dent. It has been argued that circadian rhythms in adjacent frequency bands that appear to be out of phase by 180° (i.e., 12 h) may be a consequence of temperature-dependent shifts of power from higher to lower frequencies and vice versa (16). Because the two rhythms in the waking EEG are out of phase by 75° (i.e., 5 h), they cannot be solely explained by power shifts and thus may represent distinct phenomena with different functional significance.

Circadian variation of theta activity. Previously, we suggested that the circadian variation of theta activity during wakefulness may correspond to the circadian variation in sleep propensity (4). Here, we demonstrate similarities in the timing of the two rhythms, including a gradual decrease during the daytime, a minimum in the evening, and an increase at nighttime when subjects would usually sleep. The minimum coincides with the so-called evening wake-maintenance zone (37), a phase at which the circadian signal for wakefulness is maximal (15, 17, 28, 37). A dissociation was evident as the circadian maximum of theta activity appeared to be delayed with respect to the maximum of sleep propensity. The dissociation could be a consequence of the limitations inherent in the present method of estimating circadian components in the EEG. The method is based on the assumption of an additive interaction of circadian and homeostatic components. A forced desynchrony protocol, which does not make assumptions about the type of interaction (14, 17), could be used to verify the present approximation of the wave form of the circadian modulation of theta activity.

Recent evidence suggests a causal role for melatonin in the modulation of the EEG during wakefulness. Administration of a supraphysiological dose (5 mg) of melatonin increased activity in the 5.25- to 9.0-Hz band (13). Thus it is possible that the nocturnal rise of theta activity that begins ~1 h after the onset of melatonin secretion is directly induced by the hormone. However, the present data also showed a dissociation of the circadian variation of theta activity from the melatonin rhythm: after melatonin's elimination from the plasma in the morning, theta activity did not decrease immediately. This result may imply a protracted effect of melatonin, conceivably due to residual concentrations in the brain. Alternatively, the association of onset of melatonin secretion with the rise of theta activity may represent parallel responses to a pervasive circadian signal in the evening, and the later dissociation between the two variables may reflect the absence of such a signal in the morning. In this regard, differences in

Fig. 3. Range of power density change during a 16-h waking episode, attributed to circadian and wake-dependent component. For calculation of 2 components, see METHODS and Fig. 2. Parameter estimates of circadian component (amplitude, phase) and wake-dependent component (power density value at wake-up time, asymptotic value, time constant) were used to calculate highest and lowest value of 2 components within a supposed 16-h waking episode that begins at relative clock time 0700 and ends at 2300. These values were expressed as a percentage of mean power density in each 1-Hz bin in first 16 h of CR. Plotted values are differences between highest and lowest value of each of 2 components. Values are plotted at upper limit of each 1-Hz bin. Symbols at bottom indicate significant circadian and wake-dependent components in power density during CR: ●, P < 0.01; ○, P < 0.05; 1-way ANOVA on residuals of fitted components; see METHODS.

Fig. 4. Circadian modulation of residuals of wake-dependent component in power density. Standardization of original values as for Fig. 1. Individual values were smoothed with a 3-point moving average before averaging over subjects (n = 19). A: circadian modulation of residuals in 5.25- to 6.0-Hz and 10.25- to 11.0-Hz bins. B: color-coded changes of power density residuals as a function of relative clock time and EEG frequency. Temp_min, temperature minimum.
signal strength between evening and morning hours have been postulated for the circadian signal for wakefulness (17).

Circadian variation of high-frequency alpha activity. The present data demonstrate a temporal association between the circadian variations of high-frequency alpha activity and subjective alertness. Thus, whereas theta activity may be a better correlate of objectively measurable sleep propensity, high-frequency alpha activity appears to be more related to the perception of the level of arousal. A negative rather than a positive correlation between alpha activity and alertness was reported by another group of investigators (5). The discrepancy is a result of the difference in frequency bands between their study (8–12 Hz) and the present study (10.25–13.0 Hz) and can be explained by the pronounced differences in the strength of circadian and wake-dependent components, as well as by the differences in the timing of the circadian modulation of EEG power between lower and higher alpha frequencies (see Figs. 3 and 5).

Subjective alertness has been shown to correlate with performance in vigilance tasks (22), and minute-scale fluctuations in performance in an auditory detection task have been found to covary with concurrent changes in EEG power at 10–11 Hz (32). Hence, it is likely that the circadian variation in high-frequency alpha activity also represents an electrophysiological correlate of the circadian rhythm in performance. Consistent with this idea, the circadian variations in high-frequency alpha activity in the present study and cognitive performance in a forced desynchrony protocol were similar (18). Given the striking similarities in the circadian timing, it is important to note that alertness and performance also change with increasing time awake, whereas high-frequency alpha activity does not.

The temporal association throughout the circadian cycle of changes in high-frequency alpha activity and alertness with changes in plasma melatonin and body temperature is consistent with a direct effect of one or both of the latter two variables on the former two. The
present observations, however, do not provide conclusive evidence for such a causal relationship.

Neurophysiological considerations. The present data suggest that EEG activity in different frequency bands originates from structures that are differently implicated in the regulation of sleep, wakefulness, and alertness. The neurophysiological basis of the waking EEG has been

tions are displayed by many animals during rapid-eye movement (REM) sleep and exploratory behavior and are thought to originate in the hippocampus and to be controlled by the septum/diagonal band complex as well as by brain stem structures (e.g., Ref. 40). In humans, a recent positron emission tomography study found that during a vigilance task, EEG theta activity and reaction time increased while blood flow in the medial thalamus as well as in several cortical regions decreased (33). This finding implies a connection between the thalamus, which is a key structure in the control of sleep, wakefulness, and arousal (34, 36), and EEG theta activity, which here was found to be strongly influenced by the two processes implicated in their regulation. Thalamic origin (36) and strong circadian and homeostatic influences (3, 17, 19) were previously found for spindle frequency activity in the sleep EEG. In contrast, alpha activity in the waking EEG has been

![Figure 7](image_url)

**Fig. 7.** Temporal relationship between core body temperature (A), high-frequency alpha activity (B; EEG power density in 10.25- to 13.0-Hz band), subjective alertness (C; 100 mm visual analog scales), and plasma melatonin during wakefulness (D). Data alignment as in Fig. 6. Data represent means ± SE (n = 10). Standardization as for Fig. 1. Half-hourly power densities were subjected to a 3-point moving average before averaging over subjects. Cross-correlation analysis revealed maximal negative correlations between melatonin and high-frequency alpha activity (unsmoothed residuals of wake-dependent component) at time lag 0 (r = –0.43, mean (n = 10) of individual correlations across 49 half-hourly values (1400–1400); P < 0.0001; 2-tailed t-test on Fisher’s z-transformed r values for difference from 0). Correlations between core body temperature and high-frequency alpha activity were almost equally high at lag 0 (r = 0.47; P < 0.0001) and at lag 0.5 (i.e., temperature lagging by 0.5 h; r = 0.49; P < 0.0001). Correlations between alertness and high-frequency alpha activity (unsmoothed residuals of wake-dependent component in both cases) were maximal at lag 0 (r = 0.27; P < 0.02) and at lag 0.5 (i.e., alertness lagging; r = 0.27; P < 0.01). The wake-dependent component in alertness was estimated with the same fitting procedure that was used for EEG power density (see METHODS).

![Figure 8](image_url)

**Fig. 8.** Pearson’s correlations between EEG power density and plasma melatonin (A), body temperature (B), and subjective alertness (C). Correlations were calculated separately in each subject and 1-Hz bin across 49 half-hourly values between 1400 and 1400 of CR protocol. Correlation coefficients were Fisher’s z-transformed, averaged over subjects (n = 10), and retransformed for plotting (●, ▲, P < 0.01; ○, ◆, P < 0.05; t-tests on Fisher’s z-transformed r values for differences from 0). Correlations with melatonin and body temperature were computed after removal of wake-dependent components in power density. Correlations with alertness were computed before (triangles) and after (circles) removal of wake-dependent components in alertness and power density.
related to cortical structures (31). Nevertheless, in the case of alpha activity, the thalamus also appears to play an important role, as shown in animals (30) and humans (27). A thalamic structure that receives input from the suprachiasmatic nucleus, the locus of the endogenous circadian pacemaker, is the paraventricular nucleus (see Ref. 41). This structure also shows a high melatonin receptor density (42). It remains to be elucidated, however, how a circadian signal affects thalamic structures that are specifically implicated in the generation of the EEG. Interestingly, the circadian variations in high-frequency alpha activity in wakefulness (see Fig. 7) and low-frequency alpha activity (8.25–10.5 Hz) in REM sleep (19) are similar. It is tempting to speculate that these EEG activities associated with the two activated brain states may be related to the same underlying mechanisms and neurophysiological substrates.

Kinetics of wake-dependent changes in the EEG. The present quantitative substantiates recent reports of similarities between the kinetics of the wake-dependent increase of power density in the waking EEG and sleep EEG (4, 12). The time constants for theta activity (23.9 h) and delta activity (16.6 h) in the waking EEG are comparable to the time constant that was previously derived for power density in the 0.25- to 15.0-Hz range in the sleep EEG (18.9 h; Ref. 6). The data are consistent with the hypothesis that the wake-dependent increase of these variables reflects the same underlying homeostatic process. Although the neurochemical basis of this process is unknown, it has been hypothesized to depend on adenosinergic mechanisms (7). The A1- and A2-adenosine receptor antagonist caffeine, which, according to this hypothesis, interferes with the homeostatic increase in sleep pressure during wakefulness, reduces both theta activity in the waking EEG (20, 24) and slow-wave activity (0.75–4.5 Hz) in the non-REM sleep EEG (25, 26). The distinct kinetics of beta activity in the waking EEG and the absence of unequivocal effects of caffeine on this frequency component suggest that its wake-dependent changes are related to mechanisms that are not identical to those underlying the changes of theta and delta activity.

Perspectives

It is demonstrated that during sustained wakefulness, the human EEG undergoes wake-dependent and circadian changes. With regard to the wake-dependent changes, increasing time awake is shown to be associated with an increase of power density in the delta, theta, and beta bands, whose kinetics can be described by saturating exponential functions. Sleep apparently reverses the effects of sustained wakefulness on the EEG. From these findings, we conclude that the EEG during wakefulness can be used to study the homeostatic process implicated in the regulation of sleep. With regard to the circadian changes, two distinct circadian variations in EEG activity were identified. The variations of power density in the theta band and high-frequency alpha band may represent electrophysiological correlates of different aspects of the circadian rhythm in arousal. Future studies need to be aimed at determining the nature and neurophysiological substrates of these aspects.

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