EEG and ocular correlates of circadian melatonin phase and human performance decrements during sleep loss

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Cajochen, Christian, Sat Bir S. Khalsa, James K. Wyatt, Charles A. Czeisler, and Derk-Jan Dijk. EEG and ocular correlates of circadian melatonin phase and human performance decrements during sleep loss. *Am. J. Physiol.* 277 (*Regulatory Integrative Comp. Physiol.* 46): R640–R649, 1999.—The aim of this study was to quantify the associations between slow eye movements (SEMs), eye blink rate, waking electroencephalogram (EEG) power density, neurobehavioral performance, and the circadian rhythm of plasma melatonin in a cohort of 10 healthy men during up to 32 h of sustained wakefulness. The time course of neurobehavioral performance was characterized by fairly stable levels throughout the first 16 h of wakefulness followed by deterioration during the phase of melatonin secretion. This deterioration was closely associated with an increase in SEMs. Frontal low-frequency EEG activity (1–7 Hz) exhibited a prominent increase with time awake and little circadian modulation. EEG alpha activity exhibited circadian modulation. The dynamics of SEMs and EEG activity were phase locked to changes in neurobehavioral performance and lagged the plasma melatonin rhythm. The data indicate that frontal areas of the brain are more susceptible to sleep loss than occipital areas. Frontal EEG activity and ocular parameters may be used to monitor and predict changes in neurobehavioral performance associated with sleep loss and circadian misalignment.

melatonin; sleepiness; wake maintenance zone; spectral analysis; constant routine; cross-correlation; cognitive performance; electroencephalogram; electrooculogram

SLEEP AND WAKEFULNESS as well as neurobehavioral performance during wakefulness are controlled by the interaction of an output of the circadian pacemaker, presumably located in the suprachiasmatic nuclei (SCN), and a sleep-wake-dependent homeostatic process. Desynchronization of the sleep-wake cycle from the circadian pacemaker in humans living in an environment free of time cues has revealed that the circadian pacemaker and the sleep homeostat contribute about equally to sleep tendency and alertness, and that the phase relationship between these two oscillatory processes is uniquely timed to facilitate a consolidated bout of sleep at night and a consolidated bout of wakefulness with high levels of vigilance throughout the day (16, 16a, 17). Under entrained conditions, the phase relation between the endogenous circadian rhythm and the sleep-wake cycle is such that during the 16-h waking day, stable levels of neurobehavioral function can be maintained, because the circadian pacemaker opposes the decrements in neurobehavioral function associated with increases in the homeostatic drive for sleep that accumulate with sustained wakefulness. Extension of the wake episode into the biological night, i.e., past the evening rise of melatonin, is associated with marked decrements in neurobehavioral function, because the circadian pacemaker does not oppose the wake-dependent deterioration but instead also promotes sleep at this circadian phase (17). Thus close to habitual bedtime, a sharp decrease in subjective alertness and cognitive throughput occurs. This latter phenomenon has been referred to as "the opening of the sleep gate" (29).

Various putative correlates of changes in neurobehavioral performance have been identified. In particular, eye movements and the electroencephalogram (EEG) are well-established research and clinical measures of alertness and cognitive performance. Various types of eyelid and eye movement patterns have been shown to respond to sleep loss and to correlate with sleepiness in a variety of protocols (34). Aserinsky and Kleitman (2) described slow eye movements (SEMs) during drowsiness preceding sleep onset and during light sleep. Kuhlo and Lehmann (28) found that SEMs became larger and more regularly sinusoidal when simultaneous slowing of the EEG was noted during sleep onset. Slow (0.25 Hz), pendular, horizontal eye movements were seen as the first sign of drowsiness in 50.5% of the 200 US Air Force flight personnel in a field study by Maulsby et al. (32). Furthermore, Santamaria and Chiappa (34) characterized the transition from waking to drowsiness preceding sleep onset by the disappearance of large eye blinks (EBs) and fast eye movements. Åkerstedt and Gillberg (4) quantified the effects of partial sleep loss on the incidence of SEMs and theta activity in the EEG. Other quantitative EEG studies have demonstrated associations between alertness, alpha activity, theta activity, and anterior spread of alpha activity. Furthermore, recent data indicate that changes in the EEG power spectrum in particular frequency bins accompany the fluctuations in the level of alertness, as assessed by measuring simultaneous changes in EEG and performance (26, 30). In all of these studies, the association between EEG/electrooculogram (EOG) and neurobehavioral performance was assessed at only one circadian phase. A quantitative assessment of the association between EEG/EOG parameters and neurobehavioral performance across the circadian cycle while subjects remain awake has, to our knowledge, not been reported. Such an assessment is

needed because circadian phase as well as time awake modulate both neurobehavioral performance and the EEG. It is currently not known whether the associations between EEG/EOG and neurobehavioral performance are independent of circadian phase. Furthermore, it is currently not known whether the association between ocular movements and decrements in neurobehavioral function persists when the duration of wakefulness and circadian phase are varied simultaneously. In particular, it has not been investigated whether the typical time course of neurobehavioral performance during extended wakefulness (i.e., stable levels during the first 16 h, followed by a rapid deterioration of performance thereafter) is paralleled by a similar time course in EEG and EOG parameters.

Assessment of subjective alertness and neurobehavioral performance requires subjects to be engaged in specific neurobehavioral tests at regular time intervals. Accurate assessment of neurobehavioral performance may require the use of a wide variety of neurobehavioral tests, a requirement neither met in our previous studies (17) nor in other studies describing associations between the EEG and performance (26, 30). In the present study the EEG and EOG were recorded continuously, both during and in between a variety of neurobehavioral tests, and plasma melatonin levels were assessed while subjects were kept awake for ~32 h under controlled environmental and behavioral conditions in a constant routine (CR) protocol. This protocol allows accurate assessment of endogenous circadian phase and amplitude. In addition, any parameter of interest can be followed throughout the 24-h cycle to document its amplitude and phase, as well as to measure its homeostatic regulation. The average time courses of changes in neurobehavioral performance, EEG and EOG parameters, and endogenous melatonin concentrations were computed as well as correlations between these variables. It was hypothesized that the average time course of decrements in neurobehavioral function is correlated with specific EEG and ocular concomitants and that neurobehavioral performance deteriorates during the phase of melatonin secretion.

METHODS

Subjects. Ten young men (age range 21–31 yr) were studied. Subjects were free from medical, psychiatric, and sleep disorders as determined by history, physical examination, biochemical screening tests, electrocardiograms, and psychological screening questionnaires. Subjects were instructed to abstain from caffeine, nicotine, alcohol, and drugs (including all prescription and nonprescription medications) for the two weeks before the study; their compliance was verified on the day of admission to the laboratory with urinary toxicologic analysis, which included testing for caffeine, nicotine, and common drugs of abuse. All subjects denied a history of night work or shift work in the three years before study, and none reported crossing more than two time zones in the three months before study. Subjects were instructed to keep a regular sleep-wake schedule for at least 2 wk before their admission to the laboratory. Adherence to a regular schedule during the week immediately before admission (bedtimes and waketimes within a 1-h window) was verified with a wrist actigraph (Mini Motionlogger, AMI,

Ardsley, NY, USA); only subjects who maintained the regular schedule as instructed were admitted to the laboratory for the study.

CR protocol. After three scheduled days and nights in the laboratory, during which subjects slept at their habitual times, the subjects underwent a CR as described in (13) that lasted up to 32 h. Subjects were kept awake during the CR in a semirecumbent position in dim light (<15 lux). Caloric and fluid demands were met by hourly snacks. At 1-h intervals starting 1 h after scheduled waketime, the Karolinska drowsiness test (KDT) (22) was performed, during which the subjects were instructed to relax and fixate on a 5-cm black dot 1 m away attached to a computer screen for 4 min, followed by 1 min with eye closure. The KDT either followed the neurobehavioral test battery by 15 min or preceded it by 1 h. Core body temperature (CBT) was recorded at 1-min intervals from a rectal thermistor (Yellow Springs Instruments, Yellow Springs, OH). Blood for the determination of endogenous plasma melatonin levels was sampled 2 times/h via an indwelling intravenous catheter. Samples were immediately spun, and a portion of the plasma was frozen and later assayed for melatonin concentration by RIA (assay sensitivity of 2.5 pg/ml; intra-assay and interassay coefficients of variation, 8 and 13%, respectively; DiagnosTech, Osceola WI).

EEG and eye movement recording and analysis. Bipolar EEG was derived from the z-line (Fz-Cz, Cz-Pz and Pz-Oz). The electrodes for the EOG were placed at the outer canthi of each eye, one slightly above the cantomeatal plane, the other slightly below. The left eye electrode was referenced to the electrode placed on the right mastoid (A2) and the right eye electrode to that on the left mastoid (A1). The EOG montage resembled the one commonly used in polysomnography, with the exception that the electrodes were placed somewhat closer to the eyeball. All signals were digitized on-line (12-bit AD converter, 0.122 μV/bit; storage sampling rate at 128 Hz for EEG and 64 Hz for EOG), digitally filtered at 35 Hz (4th order Bessel type antialiasing filters, total 24 dB/oct), and high-pass filtered with a time constant of 0.3 s (Vitaport-2 digital recorder; TEMEC Instruments, Kerkrade, The Netherlands). The raw signals were stored on a Flash RAM Card (SanDisk) inserted on the Vitaport-2 digital recording system and downloaded off-line to an Apple (Power Macintosh 7300/180) hard drive.

The EEG signals during the 4-min eyes-open segment of the KDT were visually inspected for EBs, SEMs, and small body movements. Two-second epochs containing muscle activity, EBs, or SEMs and microsleeps were marked as artifact and stored in a separate artifact channel (Vitaport Paperless Sleep Scoring Software, 16% of total 2-s epochs were artifacts). Artifact-free 2-s epochs were subjected to spectral analysis off-line with a fast Fourier transform (10% cosine window), resulting in a 0.5-Hz bin resolution. To enhance resolution in higher frequency bins, the raw EEG signals were prewhitened according to a squared cosine function. To fill all time intervals with an equal number of subjects ($n = 9$, EEG data from one subject were not available because of technical problems), all data were binned in 3-h intervals and z transformed. After visual inspection of the mean time course of EEG power density in each 0.5-Hz bin, EEG bands were defined from 1 to 4.5, 5 to 8.5, 9 to 12.5, and 13 to 15.5 Hz.

Different types of eyeball and eyelid movements produce characteristic waveforms depending on the characteristics of the recording system. During alert wakefulness with the eyes open, conventional EBs are of high amplitude with a short half-wave lasting ~0.2 s. Transitions from wakefulness to sleep are usually accompanied by slow, rolling, predomi-

nantly horizontal eye movements (SEMs). All EOG recordings were inspected visually, and SEMs and EBs were scored in 30-s epochs. Other eye movements (i.e., saccadic and mixed patterns) were not considered for analysis. Each 30-s epoch during the CR (~3,600 samples/CR) was scored as to whether or not at least one SEM occurred, and the presence of more than one SEM in an epoch did not influence the scoring criteria. SEMs were scored regardless of their amplitude, but SEMS that occurred during body movements or stage 1 sleep were not included in the analysis. To achieve data reduction, the percentage of 30-s epochs containing SEMs was calculated for each 5-min interval. EBs were counted per 30-s epoch during the 4-min eyes-open segment of each KDT. For subjects in whom the vertical EBs were strongly attenuated in one of the EOG channels, the EEG channel (Fz-Cz) was used in combination with the EOG to detect EBs. The average EB rate (no. per 30-s epoch) was calculated for each KDT separately.

Neurobehavioral assessment. At 30-min intervals beginning 30 min after scheduled awakening, subjects were administered a computerized neurobehavioral assessment battery of varying duration. At 30-min intervals beginning 30 min after scheduled awakening, subjects were administered computerized tests of alertness/sleepiness [Karolinska sleepiness scale (KSS) (3)] and mood. At 60-min intervals, the battery included a cognitive throughput task [addition/calculation (17)] and the KDT. Every 2 h the battery included a short-term memory task [probed recall memory (20)], simple reaction time and visual vigilance task [psychomotor vigilance test (PVT) (19, 27)], and a digit symbol substitution task.

Statistics. The statistical packages SAS (version 6.0; SAS Institute, Cary, NC) and Statistica (version 5.0; StatSoft, Tulsa, OK) were used. EEG power densities in the frequency range of 1.0–32 Hz were z transformed within each subject. All variables were binned in 1-, 2-, or 3-h intervals, averaged within these intervals per subject, and then averaged across subjects. Data were aligned with respect to each subject's habitual waketime.

One-way ANOVA for repeated measures (rANOVA) with the factor "elapsed time awake" were performed for each variable separately except for EB rate and the incidence of stage 1 sleep during the CR, for which a nonparametric ANOVA (Friedman) was used because these variables were not normally distributed. For EEG power density an additional factor, "derivation" (frontal vs. occipital), was introduced (2-way rANOVA). All P values derived from rANOVAs were based on Huynh-Feldt's corrected degrees of freedom, but the original degrees of freedom are reported. Post hoc comparisons with Duncan's multiple range test or Wilcoxon's matched pairs test were performed for EB and stage 1 sleep.

All variables for which a cross-correlation analysis was performed were binned in 1- or 2-h intervals and z transformed before entering the cross correlation. For each subject, cross correlations over 30 1-h time lags were performed. The individual correlation coefficients (r values) for each time lag interval were Fisher's z transformed before averaging across subjects. The resulting mean r values were retransformed for each time lag bin. Correlations that extended beyond ± 2 SE were considered statistically significant.

RESULTS

Time course of physiological variables. The time courses of CBT, plasma melatonin, EBs, SEMs, and epochs of stage 1 sleep during the CR are illustrated in Fig. 1*A*. The top two panels reflect the inverse relationship between the circadian modulation of CBT and plasma melatonin. EBs increased during the first 16 h of wakefulness, reaching peak values between 2000 and 2400 (relative clock time), and then dropped during subjects' habitual sleep time. The dynamics of the incidence of SEMs and stage 1 sleep were very similar to each other. Fairly stable levels were maintained during the first 16 h of the CR, with lowest occurrence of SEMs and stage 1 sleep between 2200 and 2400, when EBs were highest, CBT started to decline, and melatonin started to increase. SEMs peaked at the nadir of the CBT rhythm and declined thereafter. rANOVA revealed a significant effect of the factor "elapsed time awake" for CBT, plasma melatonin, and SEMs ($F_{14,126} = 15.2$–38.3; all $P < 0.0001$). The average duration of stage 1 sleep during the CR was 12.9 min ± SE; range was 0–49 min. The variation of EB rate failed to reach significance ($\chi^2 = 19.7$, $P < 0.13$, Friedman ANOVA).

Time course of neurobehavioral variables. The time course of subjective sleepiness (KSS), PVT, cognitive throughput, and short-term memory performance is illustrated in Fig. 1*B*. For all of these neurobehavioral variables, fairly constant levels were observed during the first 16 h after scheduled waketime, and decrements were observed thereafter. Evidence for some recovery of neurobehavioral performance was observed during the last hour of the CR, i.e., on the rising limb of the CBT rhythm. A statistical analysis revealed a significant time effect for all measures [PVT, mean reaction time: $F_{13,104} = 7.9$, $P < 0.002$; PVT, median reaction time: $\chi^2 = 57.8$, $P < 0.0001$; PVT, 10% slowest reaction time: $F_{13,104} = 5.1$, $P < 0.003$; PVT, 10% fastest reaction time: $F_{13,104} = 7.8$, $P < 0.002$; PVT, lapses (reaction times > 500 ms): $F_{13,104} = 11.3$, $P < 0.001$; cognitive performance, no. of attempts in an addition task: $F_{14,112} = 7.3$, $P < 0.001$; memory performance, no. of correct word pairs: $F_{13,104} = 3.1$, $P < 0.0009$; rANOVA or Friedman ANOVA].

Temporal correlations between neurobehavioral variables, SEMs, and melatonin. The phase relationship between the time course of subjective sleepiness, performance lapses, and 10% fastest reaction time vs. SEMs and melatonin were quantified by cross-correlation analyses (Fig. 2). One- or two-hour binned data were aligned with respect to elapsed time awake and were subjected to cross-correlation analyses. The peaks in subjective sleepiness, performance lapses, and fastest reaction time were phase locked with the maximum of SEMs [subjective sleepiness: maximum mean lag time (mean lag_{max}) = 0, $r = 0.8 \pm 0.1$; performance lapses: mean lag_{max} = 0, $r = 0.75 \pm 0.13$; fastest reaction time: mean lag_{max} = 0, $r = 0.57 \pm 0.14$].

The endogenous melatonin rhythm peaked 2–4 h earlier than the nadir of neurobehavioral variables. Maximal correlation coefficients for subjective sleepiness vs. endogenous melatonin were observed at lag −3 h (mean lag_{max} = −3, $r = 0.76 \pm 0.09$; Fig. 2). Similarly, SEMs vs. melatonin correlated maximally at −3 to −4 h (mean lag_{max} = −3 and −4, $r = 0.66 \pm 0.12$; Fig. 2), and performance lapses vs. melatonin correlated maximally at −4 h (mean lag_{max} = −4, $r = 0.66 \pm$
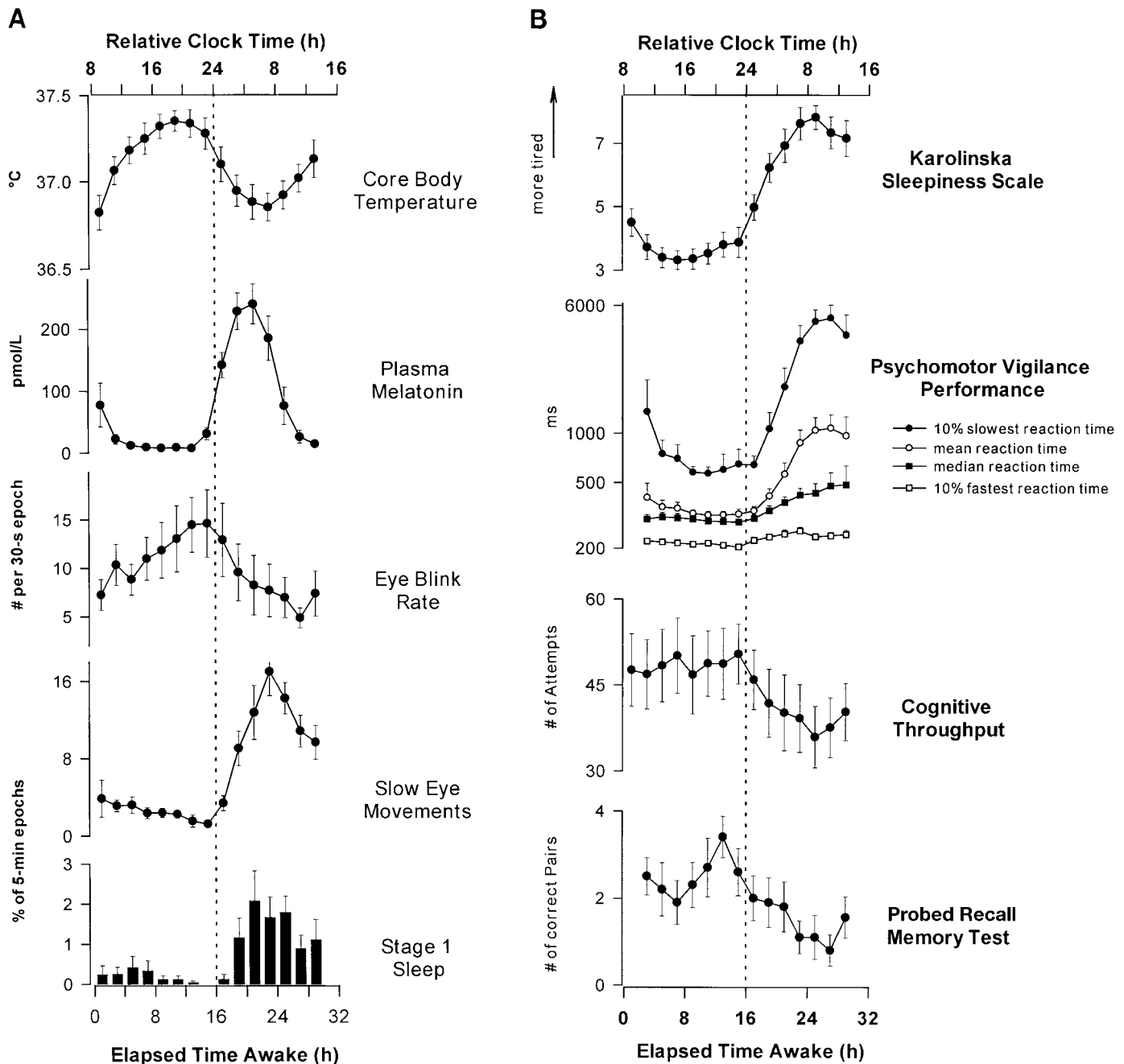
A

B

Fig. 1. *A*: time courses of core body temperature, endogenous plasma melatonin, mean eye blink rate per 30-s epoch during Karolinska drowsiness test (KDT), incidence of slow eye movements (SEMs, percentage of 30-s epochs containing at least 1 SEM/5-min interval), and incidence of stage 1 sleep (percentage of 30-s epochs containing at least 15 s of stage 1 sleep per 5-min interval) are shown, averaged across 10 subjects $\pm$ SE. *B*: time course of subjective sleepiness as assessed on Karolinska sleepiness scale (KSS; highest possible score = 9, lowest possible score = 1), psychomotor vigilance performance [mean, median, 10% slowest and fastest reaction times in ms (logarithmic scale)], cognitive performance (numbers of attempts in 4-min 2-digit addition task), and memory performance (number of correct word pairs in probed recall memory task) are shown averaged across 10 subjects $\pm$ SE. All data were binned in 2-h intervals and expressed with respect to elapsed time since scheduled waketime. Vertical reference line indicates transition of subjects' habitual wake- and bedtime.

0.13; Fig. 2), whereas fastest reaction time vs. melatonin showed highest correlation at -2 h (mean $lag_{max} = 2$, $r = 0.53 \pm 0.17$; Fig.2).

Correlation of EEG power density and neurobehavioral variables. To evaluate whether frequency-specific changes in the EEG during wakefulness are associated with changes in neurobehavioral performance for each subject, waking EEG power density in each 0.5-Hz frequency bin between 1 and 20 Hz was binned in 2-h intervals and correlated with the corresponding 2-h value of subjective sleepiness, performance lapses, and fastest reaction time (Pearson product-moment correla-
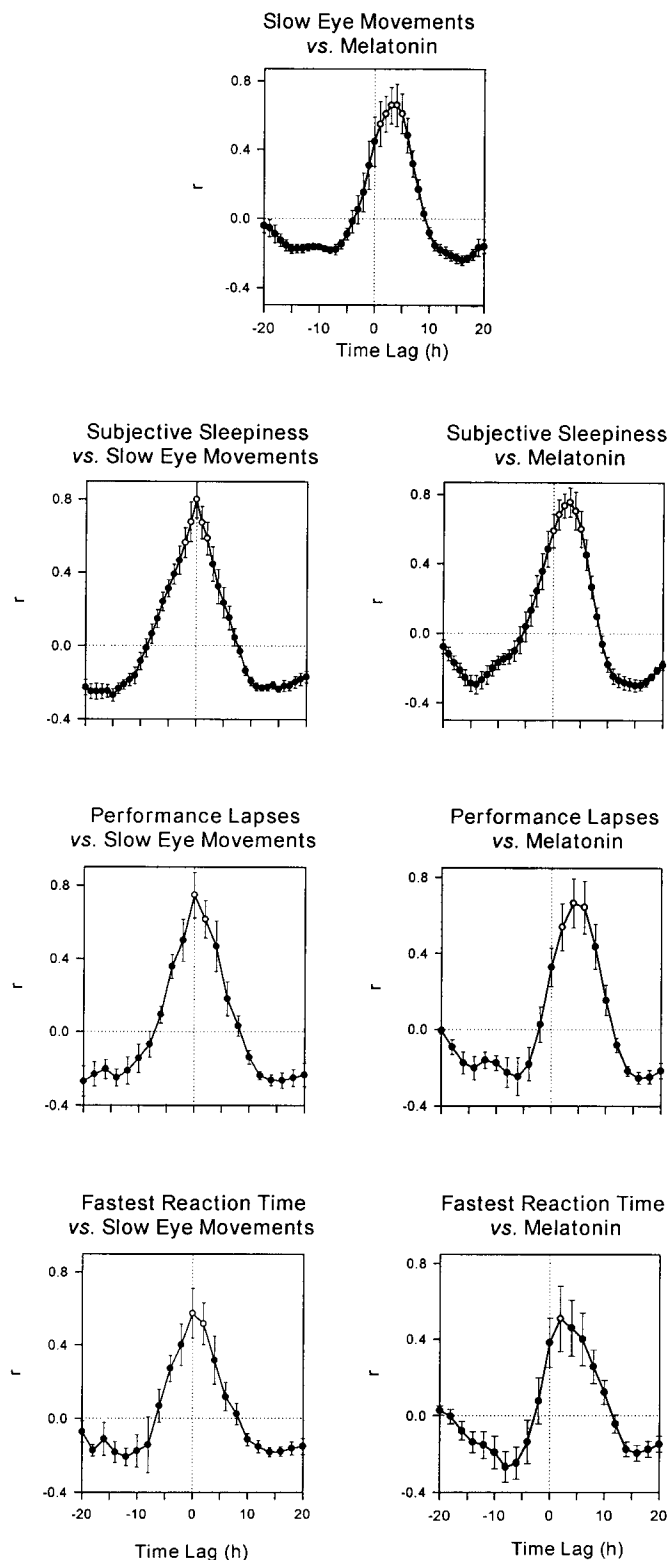
Fig. 2. Mean cross-correlation coefficients (after Fisher's z transformation; $n = 10$, means $\pm$ SE) between SEMs vs. endogenous plasma melatonin and subjective sleepiness, performance lapses, and fastest reaction time vs. both SEMs and endogenous plasma melatonin. Cross correlation at *time lag 0* represents standard Pearson correlation coefficient. Positive lags indicate a later phase than SEMs or endogenous plasma melatonin rhythm (1 lag = 1 h). Data were binned in hourly intervals for subjective sleepiness and SEMs vs. melatonin and in 2-h intervals for psychomotor vigilance variables; data are expressed with respect to elapsed time since scheduled waketime. ○, Time lags with significant correlation coefficients.
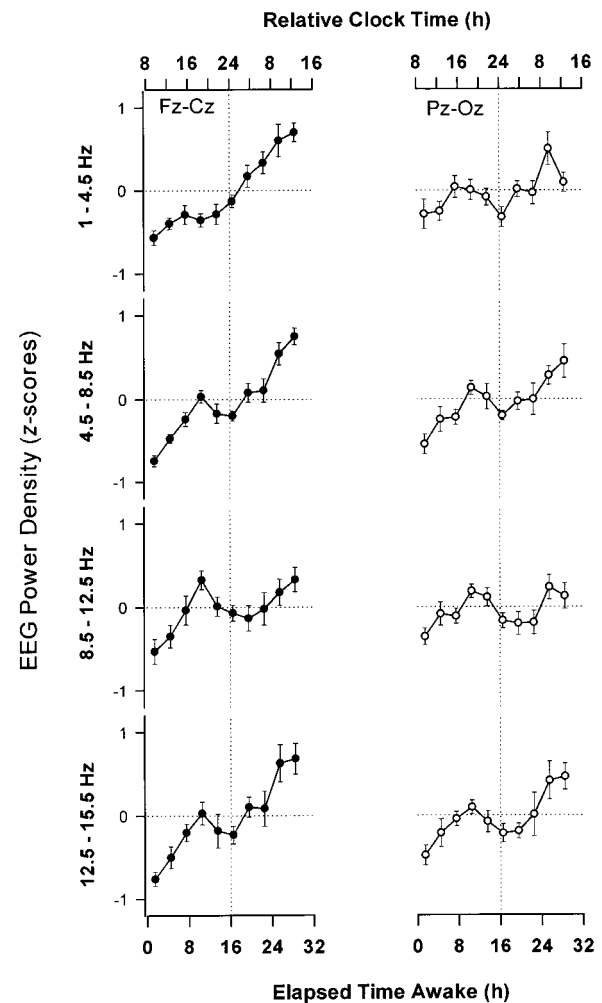

Fig. 3. Time course of frontal (Fz-Cz) and occipital (Pz-Oz) electroencephalogram (EEG) power density in different frequency bands. Before binning data in 3-h intervals and averaging over subject ($n = 9$, means $\pm$ SE), data were z transformed within each subject and expressed with respect to elapsed time since scheduled waketime and with respect to relative clock time. Vertical reference line indicates transition of subjects' habitual bedtime.

tion). The resulting correlation coefficients were Fisher z transformed before averaging over subjects, retransformed, and plotted against the corresponding frequency bin (Fig. 4). Frontal EEG power density in 0.5-Hz bins in the slow-wave and theta range (1–8.5 Hz) exhibited significant positive correlations with subjective sleepiness, performance lapses, and fastest reaction time (Duncan's multiple range test). In general, lower correlation coefficients were observed for the fastest reaction time vs. EEG power density. In addition, significant positive correlations were present for EEG power density in 0.5-Hz bins in the sigma-beta range (13–20 Hz). A similar correlation pattern was observed for the occipital derivation. In general, correlation coefficients observed for the occipital lead were lower, and EEG power density in fewer 0.5-Hz bins showed a significant positive correlation with the neurobehavioral measures: subjective sleepiness, performance lapses, and fastest reaction time.

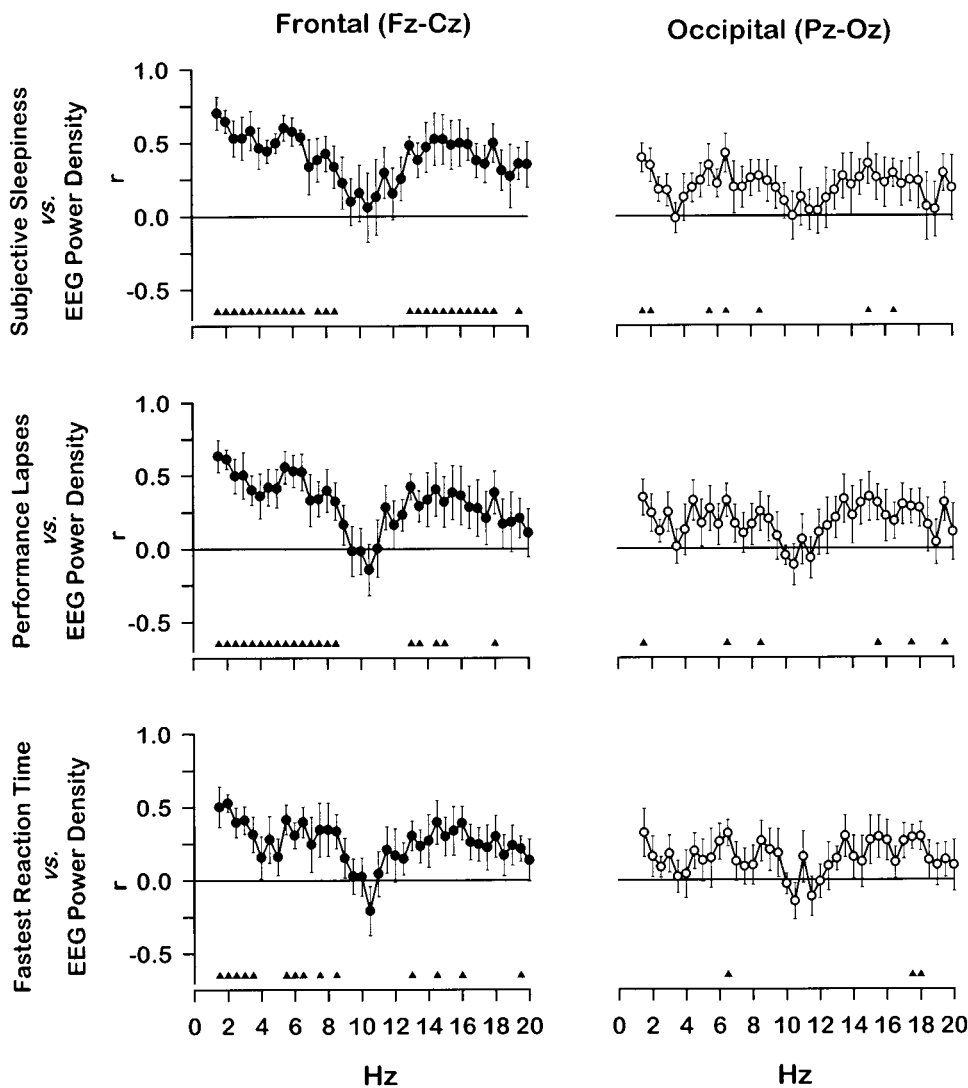*Time course of EEG power density.* EEG activity in the 1- to 4.5-Hz range exhibited a near-monotonic

Fig. 4. Pearson product-moment correlations between EEG power density during wakefulness in each frequency bin between 1 and 20 Hz (Fz-Cz and Pz-Oz) and KSS, performance lapses (reaction times > 500 ms), and 10% fastest reaction times ($n = 9$, means ± SE). ▲, significant r values ($P < 0.05$, t-test).

increase in the frontal derivation Fz-Cz (Fig. 3), with a modest local minimum during the time period from 1800 to 0100 (relative clock time). This global increase in slow EEG activity was less pronounced in the occipital derivation (Pz-Oz, Fig. 3), and the late evening decrease was more pronounced compared with the frontal derivation. This topographical difference between frontal and occipital areas was confirmed by a two-way rANOVA that revealed a significant effect of the factor "elapsed time awake" ($F_{9,72} = 9.6$, $P < 0.001$), as well as a significant interaction between the factors "derivation" and "elapsed time awake" ($F_{9,72} = 6.8$, $P < 0.003$). In addition, a significant interaction was observed for the factors "derivation" and "elapsed time awake" for EEG power density in the 4.5- to 8.5-Hz band ($F_{9,72} = 3.1$, $P < 0.02$). However, superimposed on a global increase, EEG power density in this frequency band began to decrease at 2100 and reached a trough at the beginning of the subjects' habitual bedtime at 2400, 6 h before the CBT minimum. There was no obvious global increase in EEG power density in the 8.5- to 12.5-Hz band, but there was a circadian modulation with maximal values at ~1800 and minimal values at 0300. No significant topographical differences were ob-

served (interaction between the factors "derivation" and "elapsed time awake," $P > 0.05$), whereas the factor "elapsed time awake" reached significance ($F_{9,72} = 9.5$, $P < 0.009$). EEG power density in the 12.5- to 15.5-Hz band increased throughout the CR, particularly in the frontal areas, with lower values in the late evening similar to the temporal profiles of the 4.5- to 8.5-Hz band (factor time, elapsed time awake: $F_{9,72} = 10.6$, $P < 0.0001$). On the basis of visual inspection of the curves, the circadian modulation in the occipital derivation seemed to be more pronounced. However, the interaction term derivation × elapsed time awake did not reach significance (2-way rANOVA, $P = 0.3$). Statistical analyses on the time course of EEG power density in 0.5-Hz bins revealed that the interaction term "derivation × elapsed time awake" was significant for the frequency bins from 1 to 7 Hz.

Temporal relationship between EEG power density and neurobehavioral variables. To quantify the temporal relationship between changes of frontal EEG activity in specific frequency bands and neurobehavioral performance, 2-h-binned z-transformed EEG and subjective sleepiness, performance lapses, and fastest reaction times were cross correlated for each subject sepa-
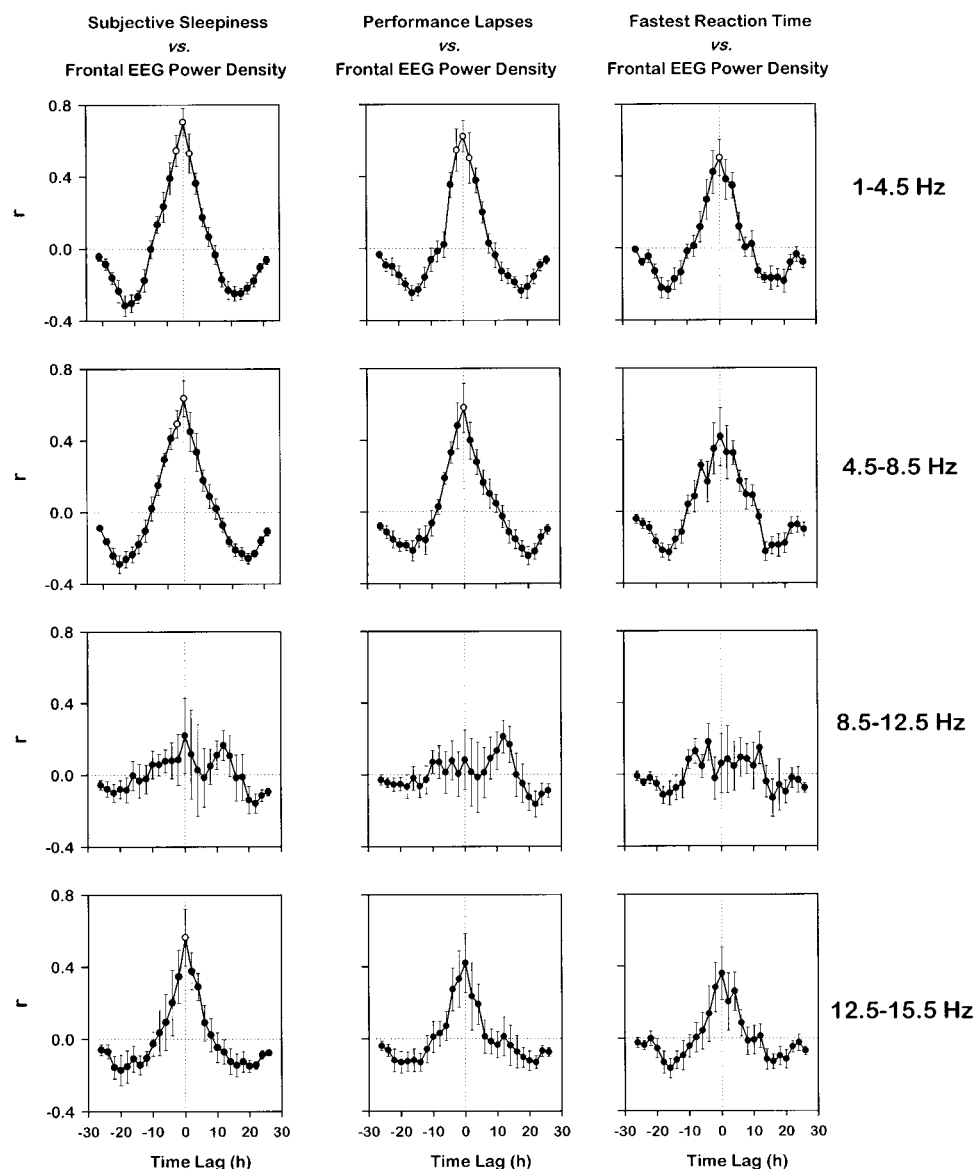
Fig. 5. Mean cross-correlation coefficients (after Fisher's *z* transformation; *n* = 8, means ± 1 SE) between frontal EEG power density in 1- to 4.5-, 4.5- to 8.5-, 8.5- to 12.5-, and 12.5- to 15.5-Hz bands vs. subjective sleepiness, and 2 measures of psychomotor vigilance performance (performance lapses and 10% fastest reaction time). Cross correlation at *time lag 0* represents standard Pearson correlation coefficient. Positive lags indicate phase delay with respect to EEG power density (1 lag = 1 h). Data were binned in 2-h intervals and expressed with respect to elapsed time since scheduled waketime. ○, Time lags with significant correlation coefficients.

rately over 15 time lags (Fig. 5). The resulting correlation coefficients were Fisher *z* transformed before averaging across subjects. Highest and statistically significant correlation coefficients between subjective sleepiness and the EEG during wakefulness were found in the frontal derivation at *time lag 0*, particularly in the slow-wave band (1–4.5 Hz), theta band (4.5–8.5 Hz), and sigma band (12.5–15.5 Hz) [subjective sleepiness, slow-wave activity (SWA): $r = 0.7 \pm 0.08$; theta: $r = 0.63 \pm 0.11$; sigma: $r = 0.57 \pm 0.14$]. Cross correlations between performance lapses and the EEG were significant at *time lag* 0 in the SWA and theta range (performance lapses, SWA: $r = 0.62 \pm 0.09$; theta: $r = 0.56 \pm 0.16$). Cross correlation between the 10% fastest reaction times and EEG power density was also highest at *time lag 0* in the slow-wave and theta range. However, only the correlation for *SWA* reached significance (fastest reaction time, SWA: $r = 0.50 \pm 0.1$; theta: $r = 0.42 \pm 0.16$). No significant correlation coefficients were obtained at any time lag for any of the

neurobehavioral measures and EEG power density in the alpha range (8.5–12.5 Hz).

DISCUSSION

The present data confirm and extend our previous observation (17) that when subjects are on a CR protocol and have no knowledge of clock time, neurobehavioral function remains at a fairly stable level throughout the 16 h that coincide with the subjects' normal waking day. Coincident with or shortly after the late evening increase in plasma melatonin, neurobehavioral function deteriorates rapidly. Objectively assessed parameters of sleepiness, such as the incidence of SEMs and short episodes of stage 1 sleep, closely paralleled these changes in subjective sleepiness and neurobehavioral performance. Although interindividual differences in the incidence of stage 1 sleep during the CR were remarkable, all subjects except one had brief episodes of sleep during scheduled wakefulness.

In addition, for each subject significant cross correlations between objectively assessed parameters of sleepiness and subjective estimation of sleepiness were found. EEG power density during extended wakefulness exhibited frequency-specific dynamics reflecting underlying circadian and homeostatic processes. In addition, frontal EEG SWA and theta activity and changes in neurobehavioral function were closely associated, although the EEG parameters exhibited a more linear time course.

Subjective sleepiness, SEMs, EB rate, and the endogenous melatonin rhythm. The loss of oculomotor control as indexed by the occurrence of SEMs was clearly related to increasing sleepiness levels, which corroborates earlier findings (2). Interestingly, EB rate tended to increase during the first 16 h of the CR protocol and dropped thereafter, just as the frequency of SEMs started to rise. The time course of EB rate was somewhat reminiscent of the inferred time course of the circadian drive for wakefulness or the alerting signal generated by the SCN, which increases gradually in the course of the subjective day, reaching a peak in the late evening hours during the wake maintenance zone (36), and then suddenly drops during the subjective night. Evidence for the prominence of this wake maintenance zone may be derived from the observation that not a single 30-s epoch of stage 1 sleep was observed between 2200 and 2400, when the duration of wakefulness was 14–16 h.

We observed a temporal relationship between the endogenous melatonin rhythm, subjective sleepiness, and the incidence of SEMs, such that subjective sleepiness and SEMs increase within ∼2 h of the onset of melatonin secretion. Similar or even closer temporal associations between the secretory phase of melatonin and sleep propensity have been observed previously under entrained as well as forced desynchrony conditions (18). In addition, several studies have demonstrated the acute hypothermic and soporific action of exogenous melatonin (for a review see Ref. 11). In a recent study by Shochat et al. (35), phase lags in sleep propensity of 100–120 min with regard to the nocturnal onset of melatonin secretion were reported. These studies suggest that when circadian phase and the duration of wakefulness are varied simultaneously, the rapid deterioration in neurobehavioral performance occurs 1–2 h after the increase in plasma melatonin levels. This contributes to the evidence for melatonin's role in regulating sleep and wakefulness. It has been suggested that endogenous melatonin secretion participates in the circadian regulation of the sleep-wake cycle and variations in alertness by inhibiting the central nervous system wakefulness generating system (9, 21, 29, 33, 36). This is supported by evidence suggesting that oral administration of melatonin in doses ranging from 0.1 to 10 mg promotes sleepiness and sleep in humans (for a review see Refs. 11, 15), and that the circadian variation of alertness during extended wakefulness is closely associated with the rhythm of melatonin (3)

EEG power density during wakefulness. The time course of power density in the EEG recorded during the KDT while subjects were fixating on a point was suggestive of both homeostatic and circadian influences on these EEG parameters. A separation and quantification of these two processes is not feasible in the present protocol, because both processes mutually interact throughout the CR protocol. However, the present data suggest that frontal EEG activity, particularly in the frequencies <7 Hz, may be primarily determined by a homeostatic process because its time course was near-linear, consistent with recent data (1, 6) and earlier reports (31). This linear time course is in accordance with the previously reported effects of sleep loss on the spectral composition of the EEG during sleep and wakefulness. In these previous studies an increase in sleep pressure was associated with a decrease or disappearance of alpha (9–12 Hz) activity and an increase in SWA (0.75–4.5 Hz) and theta activity (4.5–9 Hz) during wakefulness (4, 8) and during subsequent sleep (5).

Superimposed on the linear increase in delta, theta, and sigma activity, we observed a circadian modulation such that lower values were observed in the early nighttime hours (1800–2400), i.e., near the wake maintenance zone (36). This suggests that the strong circadian drive for wakefulness at this phase has repercussions on the EEG during wakefulness. These data corroborate recent findings (1) on the time course of EEG power density during a CR.

The prominent circadian modulation of alpha activity during wakefulness confirms earlier findings from field and laboratory studies (23). Interestingly, the nadir of the circadian rhythm of alpha activity in the waking EEG, which has also been observed in rapid eye movement (REM) sleep (18), precedes the crest of the sleepiness rhythm.

Although the changes in the EEG power spectrum were qualitatively similar in the frontal and occipital derivation, quantitative differences were apparent. In particular, the wake-dependent increase in low-frequency activity was more prominent in the frontal derivation than in the occipital derivation. We have recently obtained data to indicate that the effects of sleep loss on low-frequency components of the EEG during non-REM sleep in the subsequent recovery night may be more prominent in frontal brain areas than in posterior areas (7). This suggests that frontal areas of the brain may be more susceptible to the effects of sustained wakefulness, a hypothesis first proposed by Horne (25). Recent support for this concept may also be derived from the observation that regional cerebral blood flow in the anterior cingulate and orbitofrontal cortex as assessed by positron emission tomography is negatively correlated with EEG delta activity during sleep (24).

Changes in neurobehavioral function and the EEG spectrum. The correlation between full EEG spectra and changes in subjective sleepiness confirmed earlier and more recent reports that theta activity is associated with increased drowsiness (10, 14, 30). In addition, we observed high correlation coefficients in the SWA frequency range (1–4 Hz) and sigma activity range (14–16.5 Hz). Interestingly, our correlation spectra

strongly resembled the spectra published by Jung et al. (26), even though a different measure of performance (lapses in an auditory vigilance task) was used in that study. The cross-correlation analyses between EEG power density in selected frequency bands and psychomotor vigilance performance demonstrate that frontal EEG power density in the slow-wave and theta bands are most highly correlated with changes in performance. These high correlations were observed even though the average time course of neurobehavioral performance and EEG power density in the slow-wave range were somewhat different, in particular during the first 16 h of the CR.

Recent data using functional magnetic resonance imaging to examine brain activation in human subjects during performance of a working memory task show that the prefrontal cortex appears to play a role in active maintenance memory (keeping information available "on-line") (12). The increased SWA and theta band activity associated with reductions in performance in our data is consistent with a study showing an event-related increase of EEG amplitude in the 4- to 6-Hz range ~10 s before a performance lapse (30). In addition, the same study reported that EEG power density in the 10- to 11-Hz band is not a good predictor of lapses, and that an amplitude difference in this band appears only after the lapse. Similarly, we found very low correlation coefficients for EEG power density in the alpha range for both derivations (frontal and occipital) when correlated with either changes in performance or subjective sleepiness.

In a study by Jung et al. (26), the correlation between the EEG and an auditory monitoring task was based on the simultaneous assessment of these parameters. In our study, the interval between neurobehavioral performance assessment such as the PVT and the analyzed EEG segments was ~10–15 min. This indicates that the EEG may contain information not restricted to coincident neurobehavioral performance but may also predict deterioration in performance.

In conclusion, both EEG and EOG parameters change in the course of an episode of sustained wakefulness, and both classes of parameters correlate with neurobehavioral performance. However, the time course of EOG parameters was more similar to the time course of neurobehavioral performance than was the time course of the EEG parameters. These associations and dissociations need to be investigated under other conditions in which neurobehavioral performance deteriorates, such as chronic partial sleep deprivation (21) and forced desynchrony.

Perspectives

Further understanding of characteristic time-, amplitude-, frequency-, and topography-dependent changes in EEG activity during extended wakefulness as well as a further quantification of ocular parameters may have practical applications in the development and implementation of devices for on-line monitoring of alertness of operators of complex systems. However, before such EEG- and EOG-based systems for monitoring of alert-

ness and performance can be implemented, they need to be validated in a variety of real-life conditions. If shown to be accurate, EEG/EOG-based alertness monitoring would serve as a practical and attainable tool to not only provide on-line information about an individual's current state of alertness but also predict and prevent critical decrements in performance and alertness. The present data indicate that, in addition to information about circadian phase and the history of sleep and wakefulness monitoring, the EEG and EOG parameters can contribute to accurate prediction of neurobehavioral decrements.

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