Slow Eye Movements and Subjective Estimates of Sleepiness Predict EEG Power Changes During Sleep Deprivation

Cristina Marzano1; Fabiana Fratello, PhD2; Fabio Moroni1; Maria Concetta Pellicciari, PhD1;2; Giuseppe Curcio, PhD2; Michele Ferrara, PhD2; Fabio Ferlazzo, PhD2; Luigi De Gennaro, PhD2

1Department of Psychology, University of Rome “La Sapienza,” Rome, Italy; 2IRCCS Centro S. Giovanni di Dio, Hosp. Fatebenefratelli, Brescia, Italy; 3Department of Internal Medicine and Public Health, University of L’Aquila, Italy; 4Università Europea di Roma, Rome, Italy

Rationale: The aim of the present study was to assess, intraindividually, the relationship among slow eye movements, electroencephalogram (EEG) power, and subjective measures of sleepiness during a 40-hour sleep deprivation comparing 2 experimental conditions: eyes-open and eyes-closed.

Methods: Nineteen normal subjects participated in a sleep-deprivation protocol with recordings of the waking Cz-A1-2 EEG in 36 sessions at 1-hour intervals starting at 10:00 AM. Each session consisted of a 2-minute eyes-closed period, followed by a 4-minute eyes-open period. Electrooculogram, self-ratings (Karolinska Sleepiness Scale and Visual Analog Scale for Global Vigor), and tympanic temperature were also recorded.

Results: Changes in sleepiness and alertness are paralleled by increases in slow eye movements and theta and delta EEG power. The beginning of the rise of delta, theta, and slow eye movement activity corresponded to the nadir of temperature, peaking at 7:00 AM. Cross-correlational analyses showed that changes in slow eye movements were strictly phase locked to those in slow-frequency EEG bands and in subjective measures. The comparison of time intervals that were equivalent with respect to circadian phase confirms the effects of the increased sleepiness on slow eye movement activity and on the other measures. The temporal concordance of the different physiologic and subjective measures is also reflected in the individual time courses. Individual and group analyses converged in indicating that slow eye movements can be considered reliable indexes of sleepiness but only in the eyes-closed condition.

Conclusions: Results suggest that subjective and EEG changes associated with higher sleepiness are paralleled by an increase in slow eye movement activity, but this relationship exists almost exclusively with the eyes closed. Hence, its use in practical and operational contexts seems limited.

Keywords: Slow eye movements; sleep deprivation; subjective measures; EEG power

Citation: Marzano C; Fratello F; Moroni F et al. Slow eye movements and subjective estimates of sleepiness predict EEG power changes during sleep deprivation. SLEEP 2007;30(5):610-616

Changes in Ocular and Visual Functions During Sleep Deprivation—Including Exophoria (The Tendency of the Eyes to Diverge) and Diplopia (Double Vision)—Have Been Reported Since the Late 1930s.1,2 Currently, the significant implications of such changes in many practical and operational contexts account for several evaluations of the real effectiveness of saccadic eye-movement measures after sleep loss.3-7 Also, spontaneous slow eye movements could provide sensitive indicators of changes of alertness as a consequence of sleep loss8 and of circadian phase misalignment.9,10 The principal approach for identifying whether slow eye movements are reliable indexes of alertness consist of correlating changes in slow eye movement activity with electroencephalographic (EEG) changes,11,12 behavioral measures,9,10 and the degree of melatonin suppression.10 These data have been mostly collected in subjects with open eyes, and slow eye movements show positive, although weak, correlations with alpha EEG power spectra,13,14 theta EEG power spectra,13-15 subjective estimates of sleepiness,13,14,16 and behavioral responses.13

On the other hand, when the relationship between slow eye movements and EEG changes have been assessed in subjects with eyes closed, it has been clearly shown that, during the wake-sleep transition, slow eye movements correlate positively with EEG power in the 1- to 14-Hz frequency range and negatively with the 15- to 30-Hz frequency range.12 Therefore, the paradox is that slow ocular activity could be a valid indicator of alertness only when eyes are closed and people are already falling asleep. The different effects of the eyes-closed and eyes-open conditions in mediating the relationships among slow eye movement activity, EEG, and subjective measures have thus far not been assessed. The recording of normal subjects involved in a sleep-deprivation protocol while EEG, electrooculogram (EOG), and subjective measures were collected at 1-hour intervals makes this comparison feasible. Since any subject provides a time series of 36 (1-h binned) data points for each measure and for both the eyes-open and -closed conditions, an individual cross-correlational approach is allowed that overcomes the highly variable rate of slow eye movement activity in different people12,17 and in different alertness conditions.9

Methods

Subjects

Nineteen normal subjects (10 men and 9 women; mean age = 24.10 ± 3.36 years) were selected from a university student population.
tion to participate in the study. The requirements for inclusion were normal sleep duration and schedule; no daytime nap habits; no excessive daytime sleepiness; no other sleep, medical, or psychiatric disorder, as assessed by a 1-week sleep log and by a clinical interview. Participants were required to avoid napping throughout the experiment; compliance was controlled by actigraphic recordings (AMBI Mini-motion logger). The study on the women was carried out during the premenstrual phase of their menstrual cycle.

The subjects gave their written informed consent; the protocol of the study was approved by the local Institutional Ethics Committee and was conducted in accordance with the Declaration of Helsinki.

Design and Procedure

**General Experimental Schedule**

Each subject participated to a sleep-deprivation study across 4 consecutive days and nights. The sleep recordings, carried out in a sound-proof, temperature-controlled room, were scheduled on the first night (adaptation night), the second night (baseline sleep), and the fourth night (recovery sleep). The subjects’ sleep was undisturbed on all 3 nights and ended after 7.5 hours of accumulated sleep (as visually checked online by expert sleep researchers). Data on the sleep recordings will be reported elsewhere. Following awakening from baseline sleep, each subject participated in the protocol of 40 hours of prolonged wakefulness with 37 EEG recording sessions at 1-hour intervals starting at 10:00 AM. Each session consisted of a 2-minute eyes-closed period, followed by a 4-minute eyes-open period.

When not involved in testing sessions, subjects were allowed to carry out their own preferred activities, such as reading, writing, listening to music, watching TV, or playing games, always under the direct supervision of at least 1 experimenter. Lying down, sleeping, and vigorous physical activity were not permitted. Meals were provided to subjects at 8:30 AM, 2:30 PM, and 7:30 PM. Nonscheduled light snacks were permitted, whereas caffeinated beverages, chocolate, alcohol, and medications that can induce sleepiness were not allowed during the deprivation protocol. Time information was available to subjects, and the laboratory was constantly illuminated by 4 neon lamps (blinds attenuated the light coming from the outside). The 40-hour schedule of prolonged wakefulness ended at midnight.

**Experimental Procedure**

The waking EEG was recorded in a sound-proof, temperature-controlled room with subjects sitting on a comfortable chair in 37 sessions at 1-hour intervals starting at 10:00 AM. Each session consisted of (1) the subjective sleepiness assessment; (2) a 2-minute eyes-closed period, and (3) a 4-minute eyes-open period. During EEG recordings, subjects were asked to fixate a point on the wall or to imagine it in the eyes-closed condition.

The polysomnographic signals were continuously monitored. When signs of drowsiness were detected (e.g., closure of the eyelids in the eyes-open condition, slow pendular eye movements, and eye blinks), the subject was addressed by the experimenter and asked to respond. After each EEG recording, tympanic temperature (Braun ThermoScan thermometer) was measured 3 times from the left ear.

**Subjective Sleepiness**

Before each EEG recording, self-rated sleepiness was measured by the Karolinska Sleepiness Scale (KSS) and a Visual Analog Scale for Global Vigor. The KSS is a 9-unit rating scale, ranging between “very alert” and “very sleepy, fighting sleep”; the Visual Analog Scale for Global Vigor is a paper-and-pencil measure of subjective alertness that combines the scores on 4 continuous 10-cm scales (alert, sleepy, weary, and effort) to obtain a Global Vigor score between 0 and 40 cm.

**Polysomnographic Recordings**

An Esaote Biomedica VEGA 24 polygraph was used for polygraphic recordings. EEG signals were high-pass filtered with a time constant of 0.3 seconds and low-pass filtered at 30 Hz. Nineteen unipolar EEG channels (Fz, Cz, Pz, F3, F4, C3, C4, P3, P4, O1, O2, T3, T4, T5, T6, F7, F8, Fp1, Fp2), referenced to linked earlobes (electrode impedance lower than 5 KΩ) were recorded. Submental electromyography was recorded with a time constant of 0.03 seconds. Bipolar horizontal EOGs (time constant = 1 s) were recorded from electrodes placed about 1 cm from the medial and lateral canthi of the dominant eye. Electrode impedance was kept below 5 KΩ.

Equivalency between voltage and degree of visual angle was established through a calibration procedure with horizontal eye movements of known direction and amplitude; sensitivity was adjusted so that each degree of visual angle corresponded to 1 mm of deflection of the pens of the electrooculogram channels. For each subject, calibration was conducted in normal room lighting conditions at the beginning of the first recording session at 10:00 AM.

Any deflection of the pen equivalent to an eye movement greater than 3º and separated from another deflection by more than 200 milliseconds was considered to be an independent eye movement. Blinks, rapid eye movements, and muscle artifacts were excluded by visual inspection.

**Data Analysis**

**Polysomnographic Data Analysis**

Only EEG data from the Cz scalp derivation were used for the purposes of the present study. EEG were visually scored in 2-second epochs. This duration was chosen to maximize the duration of EEG recording free of electromyogram or EOG artifacts. The actual mean duration of waking EEG after the artifact rejection was 77.66 ± 9.84 seconds in the eyes-closed condition and 75.80 ± 14.18 seconds in the eyes-open condition (F1,18 = 0.28, P = 0.60).

Slow eye movements were scored according to a criterion of a minimal velocity of 50º per second. Slow eye movements on the horizontal EOG channel were considered if they had a duration of 1 second or longer and at least a 100-μV amplitude. The dependent measure was the percentage of time in each 20-second epoch occupied by slow eye movements. The last session (the 37th) was not considered in the analyses.

The EEG was digitized and stored on line with a sampling rate of 128 Hz. After an off-line artifact rejection, the signal from the Cz derivation was analyzed by the fast Fourier transform...
algorithm, using a 2-second resolution. Power spectra were calculated across a 1- to 30-Hz frequency range in a 1-Hz bin resolution and then averaged across the following bands: delta (1-4 Hz), theta (5-7 Hz), alpha (8-12 Hz), beta1 (13-15 Hz), and beta2 (16-24 Hz).

Mean EEG power values and slow eye movement percentages were calculated for each of the 36 sessions at 1-hour intervals starting at 10:00 AM, separately for the eyes-closed and the eyes-open periods.

Statistical Analysis

The statistical package Statistica (version 4.0; StatSoft, Tulsa, Okla) was used. EEG power values in the selected bands were z transformed within each subject. All variables were binned in 1-hour intervals, averaged within these intervals per subject, and then averaged across subjects. Data were aligned with respect to the start of the sleep-deprivation protocol. EEG power values, subjective self-ratings, and tympanic temperature were plotted in correspondence to parallel changes in slow eye movement activity.

The extent of the effects induced by the sleep-deprivation protocol have been provided by $2 \times 12$ two-way repeated-measure analyses of variance, Day (first vs second) × Hour (1, 2... 12), comparing the 2 times of day, equivalent with respect to the circadian phase (ie, the sessions between 10:00 AM and 11:00 PM on the 2 consecutive days has been highlighted in Figure 1). This analysis-of-variance design was repeated for each dependent variable of the eyes-closed and the eyes-open conditions. When significant, the means of the main effect for the factor Day and of the Day × Hour interaction were compared by 2-tailed paired t tests.

Temporal concordance between EEG and subjective measures versus slow eye movement activity was calculated by individual cross-correlations. For each subject, cross-correlations over 36 one-hour time lags were performed. The individual correlation coefficients (r values) for each time-lag interval were Fisher $z$ transformed before averaging across subjects. The resulting mean $z$ values were retransformed to $r$ coefficients for each time-lag bin.

Finally, the individual relationship among EEG power and subjective measures versus slow eye movement activity was estimated on the basis of individual product-moment correlations. Also in this case, significance was estimated after the correlations coefficients were transformed to Fisher’s $z$.

RESULTS

Time Course of the Physiologic and Subjective Variables

The time courses of EEG and subjective measures across the 36 hours in the eyes-closed (panel A) and eyes-open (panel B) conditions are illustrated in Figure 1. The time course of tympanic temperature is also reported. The concomitant courses of slow eye movement activity are superimposed.

The dynamic of the incidence of slow eye movements differs in the 2 conditions: in fact, the incidence of slow eye movements is much lower in the eyes-open condition. In both conditions, slow eye movements maintained fairly stable levels during the first 19 elapsed hours awake of the protocol (corresponding to 4:00 AM clock time), and then rose, peaking after 22 hours of wakefulness, at 7:00 AM. The increasing number of slow eye movements began in close proximity to the nadir of tympanic temperature. The peak values of slow eye movement activity coincided with the maximum subjective sleepiness and the minimal global vigor.

The dynamics of EEG delta and theta power were very similar to each other; as did slow eye movement activity, they started to increase in correspondence to the nadir of the temperature. The other EEG bands seem to be less affected by the deprivation, and the time course of alpha power (mainly in the eyes-open condition) suggests a predominant circadian modulation.

Effect of Sleep Deprivation

The $2 \times 12$ analyses of variance, Day (first vs second) × Hour (1, 2... 12), were aimed at documenting the effects induced by the sleep-deprivation protocol on the considered measures. As illustrated in the highlighted areas of Figure 1, sleep deprivation profoundly affected the EEG power bands, the slow eye movements, and the subjective measures. Table 1 summarizes the results of the analyses of variance on the means of these...
variables. The most direct influence of the deprivation is indicated by the presence of a significant main effect for the Day factor: KSS and Global Vigor, slow eye movements, and theta power show this significant effect; larger differences were found in the eyes-closed condition (ie, larger effect sizes). With respect to the effect of sleep deprivation on subjective and objective sleepiness measures, it strikingly augmented self-rated sleepiness and EEG theta power (much more in the eyes-closed condition), whereas subjective alertness dramatically decreased.

Although beyond the aims of the current study, the main effect of the Hour factor provides information on specific changes as a function of time of day, affecting the whole EEG in both conditions, and the Day × Hours interaction points to different effects of sleep deprivation as a function of the time of day. Delta EEG power showed significant effects for Hour and Day × Hour in both conditions, explained by a predominance in the second day than in the first, significant at the hour 7:00 PM (t_{18} = 2.36; P = 0.03), whereas the effects with eyes closed were explained by higher values in the second day rather than in the first, significant at the hours 10:00 AM (t_{18} = -3.79; P = 0.001) and 11:00 a.m. (t_{18} = -3.44; P = 0.003).

Similar Hour and Day × Hour effects were found in both conditions for beta2 EEG power and were explained by higher values in the second day rather than in the first day, significant at the hour 11:00 AM (t_{18} = -3.15; P = 0.005) with eyes closed and at the hours 10:00 AM (t_{18} = -3.31; P = 0.004) and 11:00 AM (t_{18} = -3.55; P = 0.002) with eyes open.

### Temporal Concordance Between Slow Eye Movement Activity and the Other Measures

The phase relationship between changes in slow eye movement activity across the 36 one-hour binned sessions and changes in each EEG or subjective variable were evaluated by cross-correlation analyses. Mean cross-correlation coefficients between slow eye movement activity versus EEG and subjective variables point to a higher temporal concordance between measures in the eyes-closed condition. Mean cross-correlation coefficients were higher in the eyes-closed condition than in the eyes-open condition, with significant relationships with delta and theta EEG bands and with both subjective measures. In more detail, the peaks in subjective sleepiness (both KSS and Global Vigor) were phase locked with the maximum of slow eye movement activity (KSS values: maximum mean lag time [mean lagmax] = 0, r = 0.42, P = 0.006; Global Vigor values: mean lagmax = 0, r = -0.42, P = 0.006). Correlation coefficients were significant at the adjacent time lags (KSS values: mean time lag = -2 [r = 0.28; P = 0.05], -1 [r = 0.36; P = 0.01], +1 [r = 0.38; P = 0.01], +2 [r = 0.36; P = 0.01], +3 [r = 0.34; P = 0.02], and +4 [r = 0.30; P = 0.04]; Global Vigor values: mean time lag = -2 [r = 0.29; P = 0.04], -1 [r = 0.39; P = 0.009], +1 [r = 0.35; P = 0.02], +2 [r = 0.31; P = 0.03], and +3 [r = 0.32; P = 0.03]).

The peaks in delta and theta EEG power values were also phase

---

**Table 1**—Summary of results from 2x12 two-way repeated measure analyses of variance, Day (1st vs. 2nd) x Hour (1, 2… 12), comparing the two times of day, equivalent with respect to the circadian phase (i.e., the sessions between 10.00 AM and 9:00 PM of the two consecutive days).

<table>
<thead>
<tr>
<th></th>
<th>Eyes closed</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P^11,18</td>
<td>P Value</td>
<td>F^11,18</td>
<td>P Value</td>
</tr>
<tr>
<td>Day</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delta (1-4 Hz)</td>
<td>2.43</td>
<td>0.14</td>
<td>2.28</td>
<td>0.01</td>
</tr>
<tr>
<td>Theta (5-7 Hz)</td>
<td>17.26</td>
<td>0.0006</td>
<td>1.85</td>
<td>0.05</td>
</tr>
<tr>
<td>Alpha (8-12 Hz)</td>
<td>2.24</td>
<td>0.15</td>
<td>2.13</td>
<td>0.02</td>
</tr>
<tr>
<td>Beta 1 (13-15 Hz)</td>
<td>0.95</td>
<td>0.34</td>
<td>1.86</td>
<td>0.05</td>
</tr>
<tr>
<td>Beta 2 (16-24 Hz)</td>
<td>0.08</td>
<td>0.78</td>
<td>2.20</td>
<td>0.01</td>
</tr>
<tr>
<td>Slow eye movements</td>
<td>51.89</td>
<td>0.000001</td>
<td>1.27</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hour</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delta (1-4 Hz)</td>
<td>1.82</td>
<td>0.19</td>
<td>2.49</td>
<td>0.006</td>
</tr>
<tr>
<td>Theta (5-7 Hz)</td>
<td>26.94</td>
<td>0.000006</td>
<td>3.28</td>
<td>0.0004</td>
</tr>
<tr>
<td>Alpha (8-12 Hz)</td>
<td>2.05</td>
<td>0.17</td>
<td>4.60</td>
<td>0.0000003</td>
</tr>
<tr>
<td>Beta 1 (13-15 Hz)</td>
<td>1.83</td>
<td>0.19</td>
<td>2.39</td>
<td>0.008</td>
</tr>
<tr>
<td>Beta 2 (16-24 Hz)</td>
<td>0.72</td>
<td>0.41</td>
<td>2.66</td>
<td>0.003</td>
</tr>
<tr>
<td>Slow eye movements</td>
<td>7.81</td>
<td>0.01</td>
<td>0.79</td>
<td>0.65</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day × Hour</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KSS</td>
<td>74.80</td>
<td>0.0000002</td>
<td>1.39</td>
<td>0.18</td>
</tr>
<tr>
<td>VAS</td>
<td>38.49</td>
<td>0.000007</td>
<td>0.89</td>
<td>0.55</td>
</tr>
</tbody>
</table>

KSS refers to Karolinska Sleepiness Scale; VAS, Visual Analog Scale for Global Vigor.
locked with the maximum of slow eye movements (EEG delta power: mean lagmax = 0, r = 0.36, P = 0.02; EEG theta power: mean lagmax = 0, r = 0.29, P = 0.04). No other time lags showed significant correlation coefficients.

The relationships between slow eye movement activity and the time courses of the other EEG bands were not significant. On the other hand, in the eyes-open condition, slow eye movements did not show any significant relation with any variable considered.

**Individual Correlations Between Slow Eye Movement Activity and the Other Measures**

The coefficients of individual product-moment correlations and their significance (2-tailed tests) are shown in Figure 2. The KSS self-ratings were significantly associated with slow eye movements in 16 out of 19 subjects in the eyes-closed condition and in 8 out of 19 in the eyes-open condition ($\chi^2$ with continuity correction: 5.54, P = 0.02). The Global Vigor scores were significantly associated with slow eye movements in 14 out of 19 subjects in the eyes-closed condition and in 7 out of 19 in the eyes-open condition ($\chi^2$ with continuity correction: 3.83, P = 0.05). The delta EEG power values were significantly associated with slow eye movements in 12 out of 19 subjects in the eyes-closed condition and in 7 out of 19 in the eyes-open condition ($\chi^2$ with continuity correction: 1.68, P = 0.19). The theta EEG power values were significantly associated with slow eye movements in 8 out of 19 subjects in the eyes-closed condition and in 5 out of 19 in the eyes-open condition ($\chi^2$ with continuity correction: 0.47, P = 0.49). The other EEG bands showed sporadic and nonsystematic associations with slow eye movement activity.

**DISCUSSION**

Prolonged wakefulness is characterized by specific time courses of oculomotor, EEG, and subjective measures as a consequence of increased sleepiness and reduced alertness. In accordance with relatively consolidated evidence,⁹,¹⁹,²⁰ changes in EEG activity are mainly expressed by parallel increases of theta and delta power, with slow-eye movements resembling this behavior. In close correspondence with the nadir of body temperature, delta, theta, and slow eye movement activity start their rise, peaking at 7:00 AM, after 22 elapsed hours of wakefulness. The comparison of time intervals, equivalent with respect to the circadian phase (i.e., from 10:00 AM to 9:00 PM before and after sleep deprivation), confirms the influence of the increased sleepiness on slow eye movement activity. Changes in slow oculomotor activity are strictly phase locked to those in delta and theta EEG activity. The temporal concordance between slow eye movements versus different physiologic and subjective measures is also reflected by the individual time courses, mainly with respect to the sleepiness and alertness changes.

This general pattern of parallel oculomotor, subjective, and EEG variations is mostly limited to the condition in which subjects are requested to stay with their eyes closed, since convergent analyses point to a greater validity of slow eye movements as indexes of increased sleepiness and decreased alertness only in the eyes-closed condition.

**Effects of Sleep Deprivation**

Analyses comparing the 2 time intervals, equivalent with respect to the circadian phase (i.e., the sessions between 10:00 AM and 9:00 PM of the 2 consecutive days), were aimed to assess the
extent of changes due to sleep deprivation in EEG, oculomotor, and subjective measures, since they allow disentangling of time-of-day and homeostatic effects. The pattern of results provides unambiguous answers with respect to the sensitivity of slow eye movements to the consequences of sleep deprivation in the eyes-closed versus eyes-open conditions: larger differences were found in the eyes-closed than in eyes-open condition. Theta power confirms its role as a biologic (EEG) marker of sleepiness, showing the largest EEG changes for the Day factor.

The other EEG bands are affected by time-of-day and/or time-of-day × homeostatic effects. In line with earlier studies using similar procedures, delta power seems a relatively weaker measure of sleepiness than theta activity; in both conditions, it was higher in the second day, with maxima in the morning hours and minima in the last hours of sleep deprivation. The existence of a floor effect should also be considered, since experimenters addressed subjects and asked them to respond when signs of drowsiness were detected.

Alpha activity exhibited a more pronounced circadian modulation with eyes open, coherent with previous observations, whereas the effects of sleep deprivation were evident only in the eyes-closed condition at specific hours. Actually, earlier studies considering the eyes-open condition also did not find significant effects of sleep deprivation or interactions with circadian components, which is in variance with findings of studies considering the eyes-closed condition.

Finally, the increase of beta1 power, in the eyes-open condition, and of beta2 power, in both conditions, at specific hours of the second day, is unexpected. Like the results of others, this finding cannot be easily interpreted. Similarly, an increase of 15- to 25-Hz EEG activity has been found in recovery sleep after sleep deprivation. These results claim a reexamination of the functional role of traditional EEG bands during extended wakefulness and sleep, also in light of the coalescence of the slow oscillation (0.5-1 Hz) with faster beta rhythms.

Eyes Closed versus Open

Slow eye movements are a distinctive feature of the wake-sleep transition. Single-cell recordings of oculomotor and premotor units during this transition in monkeys have shown that the saccade-related high-frequency discharge of burst and burst-tonic neurons decreases in peak frequency, whereas the motoneurons show a 20% to 50% drop in their firing rate as a function of eye position, and the pause neurons, which control eye fixation, become completely silent. Little is known about the presence and mechanisms of this oculomotor behavior during wakefulness with eyes open. To the best of our knowledge, only one study had the opportunity to record, in Macaca fascicularis, the electric activity of some cells in the supplementary eye field during periods of both wakefulness and drowsiness. In this study, drowsiness was shown to be associated with eyelid lowering, absence of saccades, and a significant decrease of eye velocity. Eye movements recorded with the search-coil technique actually decreased their mean velocity from 400° per second to 10° to 20° per second. The same supplementary eye field cells fired related to visually guided saccades and to slow eye movements but not in relationship to spontaneous saccades. Similarly, in human subjects involved in a motion imagery task, slow eye movements emerged repeatedly in the course of the experiment, substituting the saccades pattern. This emergence was associated with the subjective report of being drowsy and with the objective recording of lowered (but not necessarily closed) eyelids.

Hence, the appearance of slow eye movements with eyes open seems strictly dependent on the arousal state, and the cortical regions that reveal the higher sleep propensity during prolonged wakefulness (i.e., the frontal areas) are also involved in the control of these eye movements. The other side of this state-dependent occurrence is that slow eye movements are an infrequent event in wakefulness and that closing eyes makes them more likely. The actual occurrence of slow eye movements in the current study, as expressed by the time spent in this activity, was 22.21 ± 15.66 seconds in the eyes-closed condition and 4.78 ± 4.76 seconds in the eyes-open condition (F1,18 = 24.86, P < 0.0001). This should be considered in the interpretation of the current data, and a floor effect could preclude the evidence of relationships with EEG and subjective measures, limiting their validity as indexes of sleepiness in people with eyes open.

Finally, it should be mentioned that the two experimental conditions (eyes closed and open) have not been counterbalanced. This methodologic choice was made because a procedure of counterbalancing (within and/or between subjects) would have probably introduced a strong source of variation for the main dependent variable. Furthermore, a possible “sequence” effect would have affected the levels of sleepiness more than the extent of the relationship between oculomotor changes and sleepiness measures. Actually, the eye-closed condition was always characterized by a greater sleepiness, as indicated by a higher theta and delta power across the 36 hours (data not shown). Therefore, the two conditions are intrinsically characterized by a different extent of sleepiness, making this method preferable to avoid a counterbalanced design.

Phase Relationship of Slow Eye Movements Versus EEG and Subjective Changes

The peaks in slow eye movement activity at 7:00 AM (after 22 elapsed hours awake) were phase locked with the maximum of self-rated sleepiness and vigor, confirming previous results with eyes closed. Although not significant, this phase locking seemed to also affect slow eye movements recorded with eyes open. As a further original finding, the peak of slow eye movements was phase locked with the maximum of delta and theta power. Therefore, the increase of slow-frequency EEG activity was associated with a coherent pattern of increases in self-rated sleepiness and in oculomotor activity. Even though the current protocol does not allow a precise separation of homeostatic and circadian influences, the time courses across the 36 sessions and the lack of significant Day × Hour interactions are suggestive of a prominent homeostatic influence on slow eye movements and theta power. On the other hand, alpha power exhibited a circadian modulation; also for this reason, it does not show appreciable relationships with the oculomotor and subjective changes.

Does the Individual Slow Eye Movement Activity Predict Sleepiness?

With eyes closed, individual correlations point to a generalized association between slow eye movements and subjective self-rat-
ings. Although present in most subjects, this relationship seemed less robust with delta power and still less robust with theta power. Similarly, samples of slow eye movement activity, just after the start of Multiple Sleep Latency Test trials, correlate with the sleep latency in most subjects. This suggests that oculomotor activity with eyes closed may be a (although weak) predictor of physiologic correlates of increased sleep pressure.

More clearly, the possibility to use slow eye movements recorded with eyes open to predict subjective and objective correlates of sleepiness seems limited. Therefore, if sleepiness does not affect slow movements with eyes open in a predictable fashion, these changes should be cautiously considered effective and safe in their use in practical and operational contexts. However, it should also be mentioned that behavioral measures of alertness, such as the Psychomotor Vigilance Task, have not been considered in the current study and that data have been collected in a laboratory setting in which participants were involved in monotonous and boring activities. In other words, the lack of a performance demand could partly limit generalization of the present finding.

On the other hand, it should be considered that the opportunity of using this oculomotor measure in clinical settings for the assessment of daytime sleepiness, since ocular monitoring with eyes closed appears largely less demanding than measuring sleep latency in a standard Multiple Sleep Latency Test protocol.

ACKNOWLEDGMENTS

This research was partially supported by a MIUR grant to L.D.G. (Finanziamento ricerca di Ateneo 2005). Thanks to Luana Novelli, Elisa De Simoni, Lorenzo Vecchio, Francesca Feretti, Susanna Cordone, and Matteo Capozza for their help in data collecting.

REFERENCES