0021-972X/96/$03.00/0
Journal of Clinical Endocrinology and Metabolism

Vol. 81, No. 2
Printed in U.S.A.

Sleep and the Sleep Electroencephalogram across the Menstrual Cycle in Young Healthy Women*

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ABSTRACT

Cyclic changes in hormones, body temperature, and metabolic rate characterize the menstrual cycle. To investigate whether these changes are associated with changes in sleep and the sleep electroencephalogram (EEG), a total of 138 sleep episodes from 9 women with no premenstrual syndrome symptoms were recorded every second night throughout one ovulatory menstrual cycle and analyzed in relation to menstrual phase. Ovulation and menstrual cycle stage were confirmed by measurements of temperature, urinary LH, and midluteal plasma levels of estrogen and progesterone. No significant variation across the menstrual cycle was observed for subjective ratings of sleep quality and mood as well as for objective measures of total sleep time, sleep efficiency, sleep latency, rapid eye movement sleep latency, and slow wave sleep. In nonrapid eye movement sleep, EEG power density in the 14.25–15.0 hertz band, which corresponds to the upper frequency range of the sleep spindles, exhibited a large variation across the menstrual cycle, with a maximum in the luteal phase. The data show that in healthy young women, sleep spindle frequency activity varies in parallel with core body temperature, whereas homeostatic sleep regulatory mechanisms, as indexed by the time course of EEG slow wave activity are not substantially affected by the menstrual cycle. (*J Clin Endocrinol Metab* **81:** 728–735, 1996)

DURING OVULATORY menstrual cycles, prominent changes in reproductive hormones and core body temperature occur. During menstruation, concentrations of the four principle reproductive hormones, namely LH, FSH, estrogen, and progesterone, are low. Toward the end of the follicular phase, periovulation is accompanied by rises in estrogen, LH, and body temperature. In the luteal phase, concentrations of estrogen and progesterone are high, and associated with the increased progesterone, body temperature is elevated by about 0.4 C compared to preovulation values. During the luteal phase, energy expenditure (1) and sleeping metabolic rate (2, 3) are raised compared to those in the follicular phase. Along with luteal phase increases in body temperature and, therefore, energy expenditure, circulating free T_3 levels and protein metabolism, as indexed by an increase in oxidative leucine metabolism, are elevated, and it has recently been proposed that these fluctuations may be causally related (4). These physiological variations would be expected to have repercussions for the regulation of basic physiological processes such as sleep. Indeed, patients with premenstrual syndrome (PMS) or late luteal phase dysphoric disorder report insomnia, hypersomnia, unpleasant dreams, awakenings during the night, failure to wake at the expected time, and tiredness in the morning (for references, see Ref. 5). The mood symptoms in PMS occur during the late luteal phase of the menstrual cycle and remit at the early follicular (EF) phase. Sleep changes in association with the menstrual cycle may be different between women asymptomatic and symptomatic for PMS. However, in women with no symptoms of premenstrual syndrome, little or no consistent changes in sleep between the follicular and luteal phase have been observed (for reviews, see Refs. 5 and 6).

In a fairly extensive study of 8 women recorded during the menstrual cycle on 2 nights/week, no major changes in sleep structure were detected (7). In another study of 13 women recorded twice during the follicular phase and twice during the luteal phase, the only change in sleep structure consisted of a moderate reduction of rapid eye movement (REM) sleep latency in the luteal phase (8). More recently, in a group of 5 women whose sleep was recorded on 3 nights a week during a single menstrual cycle, small changes in percentages of stage 1 and REM sleep were reported, although specific values were not given (9). Although the literature indicates that no major changes in sleep structure are associated with the menstrual cycle, limitations in design and methodology may have precluded the detection of more subtle changes. Thus, in most previous studies, the data recorded during the menstrual cycle were sampled with a low resolution. By obtaining only a few recordings during the follicular and luteal phases, variations across the cycle may not have been recognized.

Moreover, with a single exception (9), sleep was only analyzed by visual inspection of the polygraphic recordings according to the standard criteria. This procedure is inade-

Received June 22, 1995. Revision August 29, 1995. Accepted September 7, 1995.

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* This work was supported by the Swiss National Science Foundation (Grant 31.32574.91), a grant from the Foundation for Scientific Research of the University of Zurich, and fellowships from the Swiss Chapter of the International Brain Research Organization (Grant 82IB-037989) and the Foundation for Research and Development of South Africa (to H.S.D.).

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quate for detecting changes in the sleep electroencephalogram (EEG). Thus, benzodiazepine and nonbenzodiazepine hypnotics induce typical changes in the sleep EEG that cannot be quantified by visual scoring (10, 11). Furthermore, gender differences in the sleep EEG were documented by spectral analysis, but were not evident in the sleep scores (12). As a final example, progressive changes in the sleep EEG spectra were recently reported to occur during pregnancy (13), which was not evident from the distribution of the visually scored sleep stages (13, 14). The recent description of sleep EEG variations in spindle frequency in association with the menstrual cycle (9) and the indication that neurosteroids may induce changes in the EEG during sleep (15) prompted us to reinvestigate sleep and the sleep EEG across the menstrual cycle. Also, to properly include ovulating women in sleep studies and to account for the menstrual phase, changes across the menstrual cycle have to be clearly documented. In the present study, the sleep stages and the sleep EEG were assessed by obtaining recordings on every second night throughout the menstrual cycle and by subjecting the sleep EEG to all-night spectral analysis.

Subjects and Methods

Subjects

Eighteen healthy women, recruited from the student population of the University of Zurich and the Federal Institute of Technology in Zurich, were selected on the basis of a questionnaire and an interview and entered a month-long screening period. The women reported having regular menstrual cycles of 24–32 days, with no menstrual-associated complaints such as menstrual pain, mood, or sleep disturbances and did not have to take time off from work because of menses. They had not taken any contraceptive or other medication for at least 4 months before the study, were nonsmokers, and were good sleepers with regular sleep-wake schedules. The General Health Questionnaire (General Practice Research Unit 1972) was used for psychological screening, and only women scoring less than 12 of 30 were included in the study. There was no indication of hyperandrogenemia, thyroid dysfunction, depression, or PMS in any of the women. Because the study was conducted over the winter months, the women were asked not to engage in activities, such as skiing, that are associated with profound changes in light exposure. The women gave their written informed consent before participating in the study, which had been approved by the local ethical committee for research on human subjects.

Screening and procedures

During the month-long screening period and for the duration of the study, the women kept a daily sleep log and were requested to maintain a regular sleep-wake schedule. Specifically, the subjects were instructed to go to bed and to get up at a the same time every day. The sleep times were individually adjusted. The subjects' rest-activity schedules were assessed by inspection of records from wrist activity monitors (Gaehwiler Electronic, Hombrechtikon, Switzerland) worn on the nondominant arm. Over weekends, the bedtimes could be shifted by an hour, but the duration of the sleep episode had to remain constant. The women were asked to refrain from caffeine intake after 1400 h and to restrict intake before this time. In the evening, the women self-rated their mood on a 100-mm visual analog scale, and listed details of exercise and intake of alcohol and medication. In the morning before getting out of bed, they measured their rectal temperature using a digital thermometer (Becton Dickinson, Basel, Switzerland). Fifteen minutes after getting out of bed, the women completed a form in which they rated their sleep quality.

Periovulatory dates were determined from both rectal temperature measurements and a commercially available kit that detects the presence of LH in urine samples (OvuQUICK, Quidel Corp., San Diego, CA; Teomed, Zurich, Switzerland). This self-test kit is an enzyme-linked immunoabsorbent assay that detects the presence of LH in urine (16). For LH assessment, urine samples were collected in the evening.

Only women who had predictable and ovulatory menstrual cycles, as assessed by a biphasic temperature rhythm and the midcycle presence of LH, and regular sleep-wake schedules, as validated by inspection of records from the wrist activity monitors, were accepted for the recording phase of the study. Nine women, aged 20–30 yr (mean ± SD; age, 24 ± 3 yr; weight, 61.7 ± 8.4 kg; height, 1.67 ± 0.05 m; body mass index, 22.2 ± 2.2 kg/m^2) from the original group of 18 fulfilled these criteria and were prepared to continue with the regular routine for the duration of a menstrual cycle during the recording phase of the study.

Recording phase

On the first night (adaptation) in the sleep laboratory, the nine women were screened for sleep apnea and periodic leg movements by all-night polysomnographic recordings including oral and nasal airflow and electromyogram from the anterior tibialis muscle.

The study was conducted over a 32- to 36-day period, during which the women spent every other night in the laboratory. They were studied in groups of two or three, and each was allocated to a private, sound-attenuated, air-conditioned room. To avoid a bias due to sequencing and acclimation effects, the women were admitted to the study at different phases of their menstrual cycle (Table 1). On the sleep-recording days, the women were asked to abstain from strenuous physical exercise and to report to the laboratory 2 h before their bedtime. They were requested to limit alcohol intake to one drink per day on nonrecording days and not to consume any alcohol on the recording days.

Lights were turned out at each woman's habitual bedtime, and recordings were made according to their habitual duration of time in bed, which ranged from 7.5–9 h. The time from lights out to lights on, ranging from 2145–0000 h and from 0600–0800 h, respectively, was kept constant for each woman. However, on seven of the recording nights there were delays in the time of lights out by 1–15 min (once only for six of the nine women and on two occasions for one woman), and on one occasion one woman went to bed and arose 20 min earlier than usual. Examination

TABLE 1. Menstrual cycle characteristics from nine women with ovulatory menstrual cycles during the screening and study periods

Subject no.	Menstrual cycle length		Ovulation	First recording night
	Screening	Study		
1	28	24	13	27 Late luteal
2	32	31	16	14 Late follicular
3	28	27	13	27 Late luteal
4	33	25	13	27 Late luteal
5	26	27	12	6 Midfollicular
6	29	30	14	21 Early luteal
7	31	39	23	8 Early follicular
8	31	32	20	9 Early follicular
9	30	28	14	11 Late follicular

The night allocated as the start of a 4-day periovulatory period (ovulation) was counted as the number of nights subsequent to the onset of menses (menstrual night 1). To avoid sequencing effects, the women entered the study at different phases of the menstrual cycle; the number of nights after the onset of menses and the menstrual phase on the first recording night are given for each woman.

of the wrist activity monitors revealed shifts in time in bed by 1–2 h in four of the seven women; three women had 1–2 h less sleep than usual (on five occasions in one woman and once each in two women), and one woman spent more time in bed (~1 h) on one morning.

Data acquisition and analysis

The EEG, submental electromyogram, and electrooculogram were recorded on Grass polygraphs (model 78E and 78D, Quincy, MA) and a portable PS1 system (Institute of Pharmacology, University of Zurich, Zurich, Switzerland). Electrodes for EEG recordings were placed at locations C3, C4, F3, and P3 and were recorded against the right or left mastoid (*i.e.* C3-A2 and C4-A1) according to the 10–20 system. Electrophysiological signals from EEG, electrooculogram, and electromyogram on the Grass polygraphs were low pass filtered [3 decibels (dB) attenuation at 27 hertz (Hz)], digitized at a sampling rate of 256 Hz, subjected to a digital low pass filter (fourth order Butterworth filter, 3-dB attenuation at 25 Hz, 24 dB/octave), down-sampled to 128 Hz, and stored on the hard disk of a personal computer. On the PS1 system, the sampling frequency was 512 Hz, and analog filters provided an attenuation of approximately 28 dB at 256 Hz. Before down-sampling to 128 Hz, the signals were filtered by a digital finite impulse response filter with an attenuation exceeding 50 dB at 64 Hz. The C3-A2 EEG signal was subjected to a spectral analysis by a fast Fourier transform routine. The power spectra were computed on-line for consecutive 4-s epochs, resulting in power density values per 0.25-Hz bins. A 10% tapered cosine window was applied. Between 0.25–5.0 Hz, the values of two adjacent 0.25-Hz bands were averaged to yield a 0.5 Hz value, and between 5.25–25.0 Hz, four adjacent values were averaged to yield a 1.0 Hz value.

The polysomnographic recordings were visually scored in 20-s epochs according to conventional criteria (17). The scorers were blind with respect to the menstrual phase of the subjects. Four-second epochs with visually detectable artifacts were identified and excluded for spectral analysis.

The power spectra for 4 s were averaged for 20 s and matched with the 20 s sleep scores. Power spectra in non-REM sleep were calculated by averaging the power density values of 20-s epochs scored as stage 2, 3, or 4. To standardize the individual power density values in non-REM sleep per frequency bin as well as the slow wave activity (SWA; mean power density in the 0.75–4.50 Hz band) and spindle frequency activity (SFA; mean power density in the 12.25–15.00 Hz band), individual values were expressed as a percentage of the corresponding mean value in non-REM sleep computed for all sleep episodes recorded.

Non-REM-REM sleep cycles, were defined according to criteria modified from those of Feinberg and Floyd (18). The non-REM sleep episodes began with stage 2 sleep; were required to contain at least 15 min of sleep stages 2, 3, and 4 sleep; and ended at the beginning of REM sleep. The last non-REM sleep episode was considered to be complete if it was terminated by REM sleep (19).

Rectal temperature was continuously recorded during the nights in the sleep laboratory. Fifteen minutes before retiring to bed, the women inserted a Yellow Springs thermistor probe (Yellow Springs Instrument Co., Yellow Springs, OH) approximately 10 cm into the rectum. The thermistor probes have an accuracy of 0.1 C within the verification range of 32–42 C. The temperature data were digitized and stored at 1-min intervals on an ambulatory monitoring system (Vitalog HMS-5000, Vitalog Monitoring, Redwood City). Ambient temperature was continuously monitored and maintained at 19–22 C.

During the recording phase, 6–8 days after ovulation, as indicated by the rise in rectal temperature and the presence of LH in urine, 10 mL blood were taken from the cubital vein. The blood samples were taken between 0800–1045 h and centrifuged, and the serum was frozen at −70 C. At the end of the study period, all blood samples were thawed and immediately analyzed in parallel for estradiol, progesterone, and PRL.

A ^{125}I RIA was used to determine estradiol (Sodiag SA, Losone, Switzerland) and progesterone (Diagnostic Products Corp., Los Angeles, CA) concentrations. Values between 180-1100 pmol/L for estrogen and between 20–90 nmol/L for progesterone were considered normal for the luteal phase. PRL was determined according to the manufacturer's recommendations with an immunoradiometric assay (Biodata, Rome, Italy). PRL concentrations between 2–20 μg/L were considered normal.

Menstrual cycle characteristics

The length of the menstrual cycles during the study period ranged from 24–39 days (Table 1). For the analysis, the menstrual cycles were normalized to 28 menstrual days, and sleep episodes were assigned to eight menstrual phases. The data were referenced to the night of the first day of menstruation and to the onset of the periovulatory period. A 4-day periovulatory window was defined by a gynecologist on the basis of morning rectal temperatures and LH in evening urine. Progesterone values higher than 20 nmol/L were interpreted as a certain indication that ovulation had occurred and indicative of a postovulatory phase. A temperature rhythm, increasing from the follicular to the luteal phase, was used to confirm the allocation of recording nights to menstrual cycle phase. Menses was defined as menstrual nights 1–4. The first night of the periovulatory period was used to allocate the three preceding nights as late follicular (LF). The three nights preceding LF were defined as midfollicular (MF). EF nights were those occurring from the fifth night after menses onset to MF. The first four nights after the four nights of the periovulatory period were early luteal (EL) and the four subsequent nights were midluteal (ML). Late luteal (LL) nights were from night 9 after the periovulatory period to the night before the onset of menses.

Statistical analysis

The number of sleep episodes contributed by each woman in the eight menstrual phases was not equal, and therefore, weighted means were calculated from the average number of sleep episodes per subject per menstrual phase. The Statistical Analysis System software program (SAS Institute, Cary, NC) was used for statistical analysis. The data for the normalized 28-day menstrual cycle were double plotted using the weighted means and SEs. For graphical representation, menstrual days were assigned such that menses was menstrual day 2, EF day 6, MF day 9, LF day 12, periovulation day 14, EL day 17, ML day 21, and LL day 25. Not all subjects contributed to the mean for each menstrual phase (Table 2), and missing data were replaced with the individual mean value across all sleep episodes. Thereafter, a repeated measures ANOVA for within-subject menstrual phase differences was calculated on the means. Statistical analysis for visually scored sleep stages were performed on a percentage of the total sleep time, whereas for the EEG power spectra in non-REM sleep, the absolute values (microvolts^2 per Hz) and percentage of the mean for each frequency bin were used. Significance levels, set at $P < 0.05$, were Huynh-Feldt adjusted, but original degrees of freedom were reported. For the first four complete non-REM-REM sleep cycles, the two-way ANOVA was calculated for the factors menstrual phase and non-REM-REM sleep cycle. For visually scored sleep variables, the duration in minutes and as a percentage of the cycle duration were entered in the ANOVA. The analysis on the EEG power spectra in non-REM sleep for sleep cycles was performed on the weighted means after log-transforming the percentage of the power density relative to each subject's mean from all nights for each frequency bin.

Results

Hormones

The nine women had normal luteal levels of estradiol (range, 190–470 pmol/L) and progesterone (range, 22.5–44.1 nmol/L). All women had normal levels of PRL (range, 7.9–21.1 μg/L). Body temperature exhibited the usual, well documented, biphasic rhythm across the menstrual cycle, with significantly elevated values during the luteal phase compared to those in the follicular phase (Fig. 1, *bottom panel*).

Subjective sleep quality and mood

Subjective assessments included estimated time to fall asleep, number of awakenings, and duration of wakefulness after sleep onset as well as ratings on sleep quality, depth of sleep, feeling restored after sleep, mood, vitality, tension, and concentration. None of the subjective assessments showed a

TABLE 2. Sleep measures across one ovulatory menstrual cycle in nine women

	ME	EF	MF	LF	OV	EL	ML	LL	Mean
TST (min)	452.8 (22.4)	454.3 (21.8)	457.6 (22.3)	444.8 (25.5)	436.3 (35.4)	449.5 (30.6)	449.8 (29.2)	452.2 (18.9)	448.9 (7.9)
SOL (min)	13.1 (5.2)	21.9 (11.3)	19.5 (5.3)	18.7 (6.5)	24.4 (24.8)	17.6 (8.8)	17.3 (10.0)	13.2 (6.5)	18.2 (2.6)
ROL (min)	66.8 (11.1)	75.0 (11.6)	75.6 (13.9)	67.2 (8.3)	63.6 (7.6)	73.1 (19.9)	76.4 (20.1)	68.7 (11.3)	69.8 (3.7)
Stg 1 (%)	5.0 (1.7)	4.2 (1.7)	5.4 (3.0)	5.4 (2.1)	4.1 (1.8)	4.4 (1.3)	4.5 (1.8)	5.4 (2.1)	4.8 (0.6)
Stg 2 (%)[a]	51.6 (4.1)	52.0 (2.3)	52.5 (4.3)	50.0 (4.2)	51.9 (3.1)	55.3 (4.3)	53.3 (3.1)	52.6 (2.9)	52.1 (1.0)
Stg 3 (%)	5.2 (1.9)	4.5 (0.7)	4.3 (1.1)	5.2 (2.3)	6.0 (2.3)	5.4 (2.1)	5.8 (1.9)	5.8 (1.9)	5.4 (0.6)
Stg 4 (%)	12.6 (3.7)	11.9 (2.7)	12.3 (4.6)	13.0 (2.4)	12.1 (3.0)	11.5 (3.2)	12.0 (3.7)	13.3 (3.1)	12.5 (1.0)
SWS (%)	17.8 (5.0)	16.4 (3.3)	16.6 (4.9)	18.2 (4.3)	18.1 (5.0)	16.8 (5.0)	17.8 (4.1)	19.1 (4.4)	17.8 (1.5)
REM (%)	25.7 (2.6)	27.4 (1.9)	25.6 (2.8)	26.5 (2.6)	25.9 (2.4)	23.5 (1.9)	24.5 (3.1)	22.9 (3.6)	25.3 (0.9)
WASO (%)	1.8 (1.5)	1.8 (1.2)	2.0 (2.3)	3.7 (3.6)	4.8 (5.4)	2.7 (3.1)	3.0 (2.4)	3.2 (2.8)	2.8 (0.7)
MT (%)	2.5 (0.8)	2.5 (0.8)	2.9 (0.8)	2.5 (0.9)	2.4 (0.7)	2.9 (0.7)	2.4 (0.7)	2.3 (1.0)	2.5 (0.3)
SE (%)	93.3 (1.6)	91.7 (3.3)	91.7 (2.9)	90.7 (3.0)	89.1 (7.2)	91.5 (3.5)	91.6 (3.6)	92.4 (2.7)	91.6 (0.9)
n-epi	23	20	10	18	18	16	17	16	138
n-subj	9	7	8	9	9	8	9	8	9

ME, Menses; EF, early follicular; MF, midfollicular; LF, late follicular; OV, ovulation; EL, early luteal; ML, midluteal; LL, late luteal. Values for the eight menstrual phases represent weighted means (SD). For the total mean (SEM) over the menstrual cycle, a mean from all sleep episodes was calculated per subject and then averaged across subjects. TST, Percentage of total sleep time; SOL, sleep onset latency; ROL, REM sleep onset latency; Stg, stage; SWS, slow wave sleep; WASO, wakefulness after sleep onset; MT, movement time; SE, sleep efficiency; n-epi, number of sleep episodes; n-subj, number of subjects contributing to the means.

[a] By repeated measures ANOVA, $P \leq 0.007$.

significant variation over the menstrual cycle (data not shown).

All-night sleep measures derived from polysomnograms

Polysomnographically assessed total sleep time (TST), latency to sleep onset (first epoch of stage 2 non-REM sleep), REM sleep latency (taken from sleep onset), and sleep efficiency (TST/time in bed) did not vary significantly over the menstrual cycle (Table 2). REM sleep, expressed as a percentage of TST (percent REM sleep), tended to be higher in EF than in LL (by repeated ANOVA, $P = 0.081$), and the percentage of non-REM sleep (stages 2, 3, and 4) showed a significant menstrual cycle rhythm, with higher values in the luteal compared to the follicular phase (by repeated ANOVA, $P = 0.04$; Fig. 1). The variation in non-REM sleep was largely due to the percentage of stage 2 sleep, which increased from the lowest value in LF to the maximum in EL (Table 2). The time spent in slow wave sleep (stages 3 and 4; see Table 2) did not vary significantly over the cycle and neither did SWA in non-REM sleep (Fig. 1). The percent non-REM sleep and SFA in non-REM sleep varied in parallel with the temperature rhythm, showing high values in the luteal phase and low values in the follicular and ovulatory phases (Fig. 1).

All-night EEG measures derived from spectral analysis

The effect of menstrual phase on EEG power density in non-REM sleep in the frequency range from 0.25–25.0 Hz is shown in Fig. 2. Large changes were only present in the range of sleep spindles and the adjacent higher frequency bins (13.25–18.0 Hz), with maximum variation in the 14.25–15.0 Hz band. Power density in this frequency range was lowest in the MF phase and highest in the ML phase. Minor variations were present in the θ- to α-band (7.25–9.0 Hz) as well as in the 17.25–18.0 Hz band. In these frequency ranges, there was a significant effect of menstrual phase on the percentage of the mean, but when computed on the absolute values (microvolts^2 per Hz), these variations did not reach statistical significance. The maximum power density in the θ-band was observed in the ML phase, and the minimum during ovulation.

Time course of sleep measures and body temperature across sleep episodes

The time course of selected sleep measures and rectal temperature during sleep episodes in different phases of the menstrual cycle is shown in Fig. 3. Sleep data were computed for the first four non-REM-REM sleep cycles. A two-way

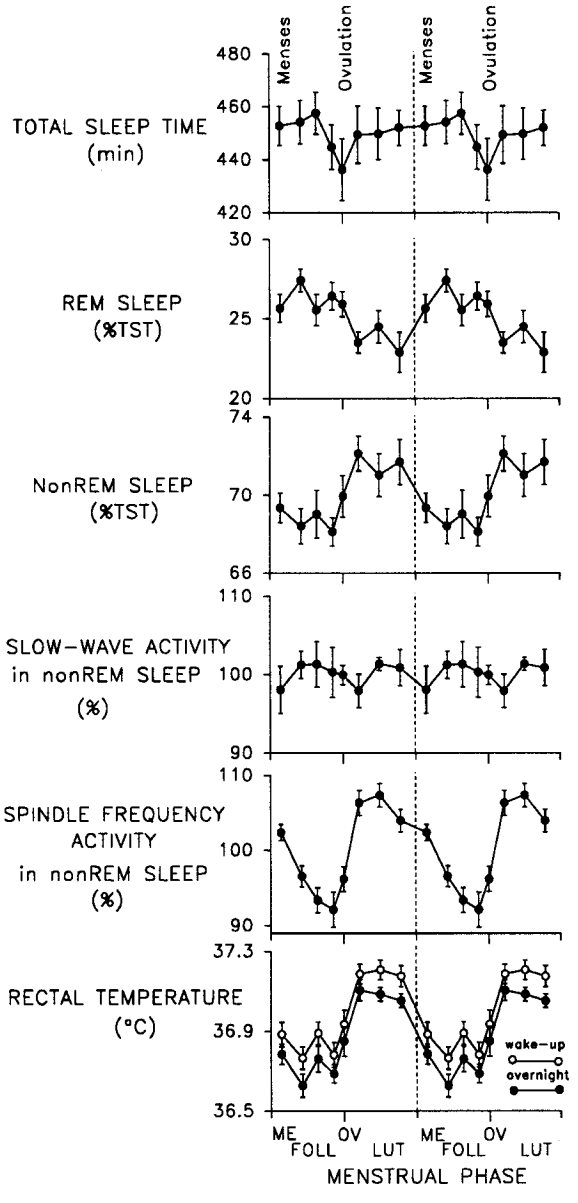

FIG. 1. Sleep, sleep EEG variables, and rectal temperature double plotted for a normalized 28-day menstrual cycle. The *curves* connect the weighted mean values (± 1 SEM) of nine women. The *vertical dotted line* delimits the junction between menstrual cycles. The *bottom panel* shows the mean rectal temperatures during the sleep episode (overnight) and after awakening in the morning (wake-up). Selected sleep measures include the total sleep time, percentage of REM and non-REM (stages 2, 3, and 4) sleep, SWA (power density in the 0.75–4.50 Hz range), and SFA (12.25–15.0 Hz) for EEG power density in non-REM sleep. Repeated measures ANOVA revealed a significant menstrual phase effect on rectal temperature, non-REM sleep, and SFA ($P \leq 0.05$). ME, Menses; FOLL, follicular; OV, periovulation; LUT, luteal.

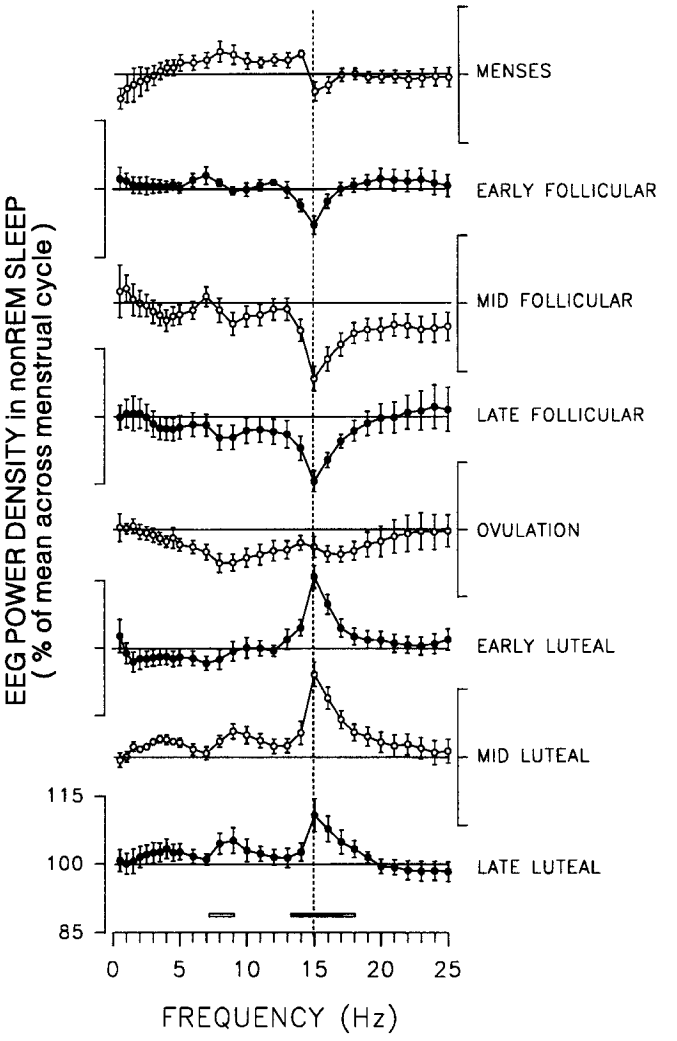

FIG. 2. Changes in EEG power density in non-REM sleep over the menstrual cycle of nine women. The mean power density combining all nights was calculated per frequency bin for each woman, and the percentage of the mean in each of eight phases of the menstrual cycle was obtained. The mean power density for all nights was 100%. The weighted means of the percentage of the mean were plotted using the same scale for the eight panels, such that symbols represent the upper end of each frequency bin. The predominant menstrual phase change was in the 14.25–15.0 Hz bin, indicated by the *vertical dotted line*. *Horizontal bars at the bottom* of the figure represent the frequency range across which significant menstrual phase differences occurred based on repeated measures ANOVA [■, $P < 0.002$ on percent and absolute values (microvolts^2 per Hz); □, $P < 0.02$ on percent values only].

ANOVA with factors sleep cycle and menstrual phase yielded significant effects of sleep cycle on all sleep measures except time awake (not shown). Significant effects of the factor menstrual phase were observed for non-REM-REM sleep cycle duration, time (minutes) spent in non-REM and REM sleep, and SFA in non-REM sleep. The mean non-REM-REM sleep cycle durations from all nights were averaged and ranged from 69–122 min, with a mean of 96 min. In the EF phase, the mean non-REM-REM sleep cycle duration was 104 ± 16 min compared to 89 ± 17 min in the EL phase. When expressed as a percentage of the non-REM-REM sleep cycle duration, only REM sleep varied significantly over the menstrual cycle. As in the analysis of TST, higher values of REM sleep occurred in the follicular than the luteal phase (Fig. 3). Although the percentage of REM sleep increased from cycle 1 to cycle 4 and the percentage of non-REM sleep decreased, similar amounts of both were present in the second and third sleep cycles, except in EF and LL. The typical monotonic decline in SWA from the first to the fourth sleep cycle was

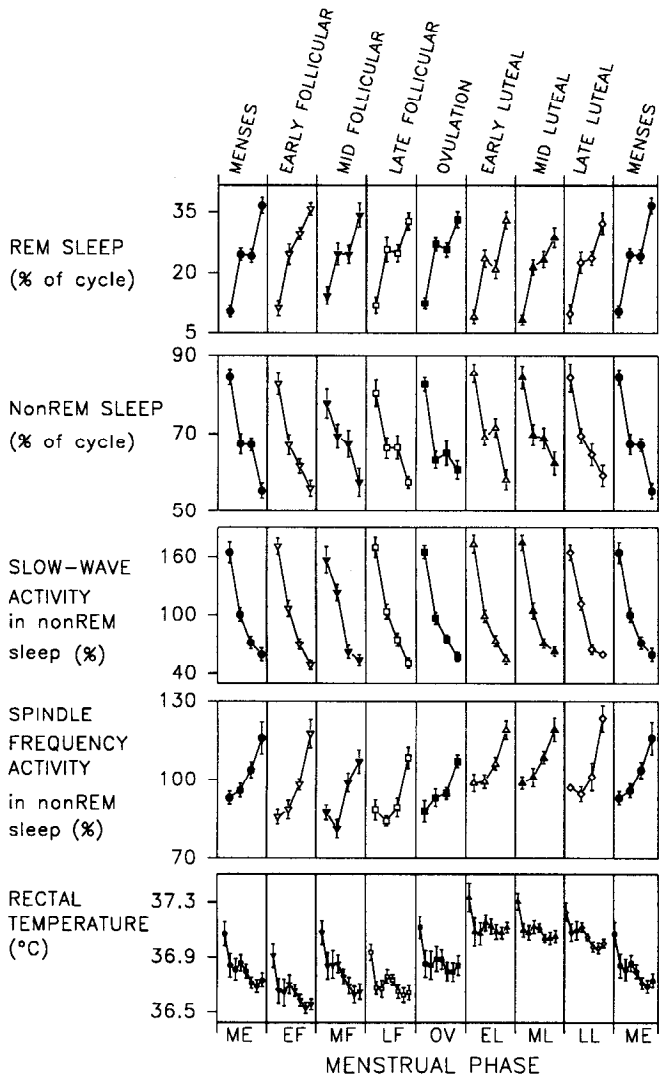

FIG. 3. Changes in REM and non-REM sleep, SWA, and SFA (in non-REM sleep) during the first four non-REM-REM sleep cycles as well as hourly temperature at eight phases of the menstrual cycle. Data for nine women were plotted from menses to menses. *Symbols* represent weighted means, and *bars* represent SES. SWA and SFA were calculated as a percentage of the mean for all nights. Core body temperature data were calculated at lights out and then at 1-h intervals over the next 7 h. The repeated ANOVA for the factor sleep cycle was significant for all sleep measures, whereas the factor menstrual phase was significant for percent REM sleep and SFA ($P < 0.05$). There was no significant interaction for the factors menstrual phase and sleep cycle.

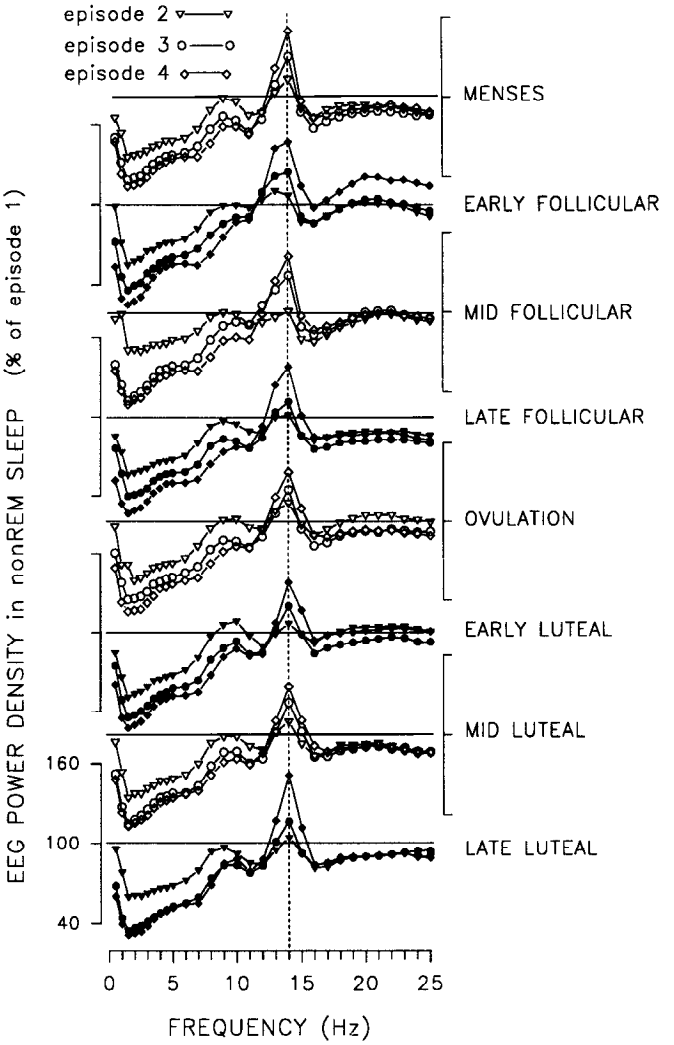

FIG. 4. The time course of EEG power density in non-REM sleep episodes over the first four non-REM-REM sleep cycles of nine women at eight phases of the menstrual cycle. Weighted means, expressed as a percentage of the values in the first non-REM sleep episode (100%), were plotted for the second, third, and fourth episodes according to menstrual phase and using the same scale for all panels. The two-way ANOVA for the factor sleep cycle was significant in all frequency bins ($P < 0.05$), whereas significant changes according to menstrual phase were in the 3.75–10.0 and 13.25–19.0 Hz regions. The predominant sleep cycle effect was in the 13.25–14.0 Hz spindle frequency region indicated by the *vertical dotted line* at 14.0 Hz. Only in the range from 19.25–20.0 Hz was a significant interaction for the factors menstrual phase and sleep cycle found ($P < 0.05$).

observed during all phases of the menstrual cycle. SFA increased across non-REM-REM sleep cycles, although a rise from the first to the second value was not present in all phases.

Rectal temperature was represented by the value at lights out, and thereafter, hourly average values were computed for the first 7 h. ANOVA revealed a significant effect of time (hours) and menstrual phase. In all phases of the menstrual cycle, the largest drop in nocturnal temperature occurred during the first hour after lights out. The modulation of the temperature level by the menstrual cycle is apparent. In addition, the time course of temperature during the sleep episode was flatter at ovulation and during the luteal phase than during the follicular phase. Neither the sleep variables nor body temperature showed a significant interaction between the factors menstrual phase and sleep cycle or time.

Time course of EEG power density across sleep episodes

Figure 4 illustrates the changes in EEG power density in non-REM sleep over the first four non-REM-REM sleep cycles at eight phases of the menstrual cycle. The values for the second, third, and fourth non-REM-REM sleep episodes are expressed relative to the value of the first episode (100%). The typical decline in power density in the low frequency

range and the increase in the spindle frequency range were present in all menstrual phases. There was a significant effect of the factor sleep cycle in all frequency bins. The largest sleep-dependent effect in the spindle region was observed at 13.25–14.0 Hz. An effect of the factor menstrual phase was observed for the frequency ranges from 3.75–10.0 Hz and from 13.25–19.0 Hz. The only interaction between the factors menstrual phase and sleep cycle was observed in the 19.25–20.0 Hz bin.

Discussion

This is the first systematic study of sleep during the menstrual cycle. The results reveal that cyclical hormonal and body temperature changes during ovulatory menstrual cycles are associated with a degree of variation in particular sleep measures, with little variance in some others. The variables that did not exhibit significant changes included subjective sleep quality, objective total sleep time, sleep efficiency, sleep onset latency, and EEG SWA in non-REM sleep. These results are consistent with previous findings (7–9). The most salient finding of our experiment was the prominent variation of EEG power density in the 14.25–15.0 Hz band across the menstrual cycle.

The absence of significant changes in sleep latency and sleep efficiency indicates that the hormonal and metabolic changes during the menstrual cycle are not associated with major changes in sleep propensity and sleep continuity. The absence of an effect on the time course of SWS or SWA is in accordance with the results from previous studies (7–9). Moreover, the consistency of SWS and SWA across the menstrual cycle is reminiscent of the invariance of these sleep variables across the 24-h cycle. Thus, when sleep was initiated at various phases of the circadian rhythm, the time course of SWS and SWA remained constant (20, 21). As SWA and its time course during sleep are considered to be primary indicators of sleep homeostasis (22, 23), the results indicate that this facet of sleep regulation is hardly affected by menstrual phase. However, some caution is indicated in interpreting the present data, because the time in bed was restricted, and minor changes in sleep propensity and sleep need may not have been observed.

REM sleep underwent minor, yet significant, variations across the menstrual cycle. Its fluctuation of 4.5% (from 27.4% in EF to 22.9% in LL) and its overall mean level of 25.3% were similar to the mean of 27.5% reported by Parry *et al.* (7) and 26% reported by Williams *et al.* (24) for women in the follicular phase. The low percentage of REM sleep (20%) in the study of Lee *et al.* (8) may be related to the negative premenstrual affective symptoms that were present in more than half of their subjects. The shortened REM sleep latency during the luteal phase observed by Lee *et al.* (8) could not be confirmed in the present study. The percentage of REM sleep plotted across the menstrual cycle was inversely related to rectal temperature. A similar relationship was seen during the night when the rising level of REM sleep was associated with the decline in temperature. It is well known that circadian variations of REM sleep and body temperature are inversely related; the maximum REM sleep propensity is close to the minimum core body temperature (21, 25). It, therefore, seems reasonable to conclude that the variations in REM sleep associated with the menstrual cycle are related to the processes that modulate body temperature. Whether these processes also influence the non-REM-REM sleep cycle duration, which is longer in the EF than in the early luteal phase, remains to be investigated.

The main new finding of this study was the prominent variation in stage 2 sleep and in the sleep EEG in the 14.25–15.0 Hz band that corresponds to the upper frequency range of sleep spindles. Power density in this and the adjacent frequency bands reached a maximum in the ML phase and decreased to a minimum in the MF phase. These EEG changes in SFA, which were limited to non-REM sleep, paralleled changes in rectal temperature. In a previous study, sleep spindle measures (*i.e.* density, amplitude, and duration) showed no change over the menstrual cycle; only spindle frequency exhibited a small (*i.e.* 0.05 Hz), but significant, variation (9).

Two studies on sleep changes during the menstrual cycle included women who were symptomatic for PMS and reported increased stage 2 sleep compared with controls (7, 8). Seven of a group of 13 ovulating women between 25–35 yr of age were classified as being premenstrually symptomatic (8). The women with PMS demonstrated less SWS (an average of 4.4 ± 2.5% compared to 15.5 ± 5.1%) and more stage 2 sleep during both menstrual cycle phases compared with asymptomatic women. Although the symptomatic group had an average of 7–10% more stage 2 sleep during both phases of the menstrual cycle than the asymptomatic group, the difference was only significant in the follicular phase. More stage 2 sleep (average, 10%) was also found in 8 women, between 26–45 yr of age, who met diagnostic criteria for PMS, compared with 8 control women (7). However, in this group of women with PMS, SWS time was similar, whereas, in contrast to the women in the study by Lee *et al.* (8), REM sleep was decreased to a mean of 21.7% compared to 27.5% in the control women (7). Although neither of these two studies found a phase effect on stage 2 sleep in the control groups, we found increased stage 2 sleep and a striking increase in SFA in the luteal compared with the follicular phase. These luteal effects on stage 2 sleep and SFA may be exacerbated and persist into the follicular phase in women with premenstrual symptomatology and deserve further investigation.

Within the frequency range of sleep spindles, the observed variation in power density may reflect the menstrual phase fluctuation in and the actions of progesterone or related neurosteroids. The neurosteroids appear to bind to a single site on the γ-aminobutyric acid$_A$ (GABA$_A$) receptor and act as allosteric agonists or inverse agonists by modulating the frequency and duration of chloride channel openings (for review, see Ref. 26). During pregnancy, a profound reduction in power density in the frequency range of 14.25–15.0 Hz has been reported (13). Recently, the neurosteroid pregnenolone, an inverse GABA$_A$ receptor agonist, has been shown to reduce EEG σ power (10.26–14.1 Hz) (15). These data indicate that EEG activity within the frequency range of sleep spindles is susceptible to changes in the levels of reproductive hormones and neurosteroids. However, it should be noted that the enhancement of SFA in the sleep EEG, which is a typical effect of benzodiazepine hypnotics and their analogs

(10), differs from the variations observed in the course of the menstrual cycle. The maximum drug-induced change occurred between 12.25–13.0 Hz, 2 Hz below those associated with the menstrual cycle. Thus, if both types of changes are mediated by the $GABA_A$-benzodiazepine receptor complex, different binding sites must be invoked. For a better understanding of these phenomena, a more detailed analysis of sleep spindle activity in terms of both regional distribution and frequency is needed.

Power density in the spindle frequency range varied across the menstrual cycle and within the sleep episode. In the present and previous studies (19, 27), the sleep-dependent changes were most pronounced in the 13.25–14.0 Hz range. In contrast, the changes associated with menstrual cycle phase were located in the 14.25–15.0 Hz range, which suggests a specific effect due to cyclical hormonal release, as reflected by the progesterone-mediated biphasic menstrual temperature. Similar differential effects on power density in adjacent frequencies within the frequency range of sleep spindles have been described across the circadian cycle (28).

Spectral analysis of the sleep EEG revealed minor, but statistically significant, changes in non-REM sleep in the frequency range from 7.25–9.0 Hz. The highest power density values were observed in the luteal phase. This contrasts with changes in α activity during wakefulness, recorded at the same time of day every other day over a menstrual cycle, which were higher during the follicular phase than in the luteal phase, when α frequencies were higher (29).

In conclusion, the present data show that homeostatic sleep regulatory mechanisms, as indexed by the time course of SWA, sleep onset latency, and sleep efficiency, are insensitive to the marked changes in the *milieu interieur* associated with the menstrual cycle in healthy women. Small fluctuations in REM sleep are inversely related to biphasic menstrual changes in body temperature. During the luteal phase, when progesterone levels are high, the EEG spectrum in non-REM sleep in the range of high frequency sleep spindles showed a maximum. These changes may reflect variations in neurosteroids or other hormones acting on the brain and demonstrate that the sleep EEG is a highly sensitive indicator of subtle repercussions of the menstrual cycle on central nervous system functions.

Acknowledgments

We thank the women who participated in the study, Hans-Peter Landolt for help with the data acquisition, Beat Geering and Jörk Pischke for soft- and hardware development, and Drs. Peter Achermann, Rochelle Buffenstein, and Irene Tobler for comments on the manuscript. The loan of Vitalog equipment by Mr. P. Kocher, TechMed (Zurich, Switzerland), is gratefully acknowledged.

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