

# Circadian temperature and melatonin rhythms, sleep, and neurobehavioral function in humans living on a 20-h day

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**Wyatt, James K., Angela Ritz-De Cecco, Charles A. Czeisler, and Derk-Jan Dijk.** Circadian temperature and melatonin rhythms, sleep, and neurobehavioral function in humans living on a 20-h day. *Am. J. Physiol.* 277 (Regulatory Integrative Comp. Physiol. 46): R1152–R1163, 1999.—The interaction of homeostatic and circadian processes in the regulation of waking neurobehavioral functions and sleep was studied in six healthy young subjects. Subjects were scheduled to 15–24 repetitions of a 20-h rest/activity cycle, resulting in desynchrony between the sleep-wake cycle and the circadian rhythms of body temperature and melatonin. The circadian components of cognitive throughput, short-term memory, alertness, psychomotor vigilance, and sleep disruption were at peak levels near the temperature maximum, shortly before melatonin secretion onset. These measures exhibited their circadian nadir at or shortly after the temperature minimum, which in turn was shortly after the melatonin maximum. Neurobehavioral measures showed impairment toward the end of the 13-h 20-min scheduled wake episodes. This wake-dependent deterioration of neurobehavioral functions can be offset by the circadian drive for wakefulness, which peaks in the latter half of the habitual waking day during entrainment. The data demonstrate the exquisite sensitivity of many neurobehavioral functions to circadian phase and the accumulation of homeostatic drive for sleep.

circadian rhythms; performance; body temperature; alertness; memory

IT IS GENERALLY ACCEPTED that the endogenous circadian pacemaker, located in the suprachiasmatic nucleus of the hypothalamus, plays a pivotal role in the regulation of sleep propensity, sleep structure, and waking neurobehavioral function. It has been established that in addition to this circadian process, a homeostatic process that tracks the duration of wakefulness and sleep contributes significantly to sleep propensity and waking neurobehavioral function (15, 18). Many protocols have been applied in the effort to measure the interaction of the circadian and homeostatic processes and their contribution to the variability of human sleep and waking neurobehavioral performance, with the ultimate goal of constructing sufficiently accurate predictive models (1, 5, 15, 33). Quantification of this interaction requires that measurements of sleep propensity and waking neurobehavioral function be obtained

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for a wide range of the two independent variables, i.e. circadian phase and duration of prior wakefulness.

Sleep fragmentation (4), partial or selective sleep deprivation (28, 51), total sleep deprivation (3, 24, 27, 30), and constant-routine protocols (34) have the benefit of varying levels of homeostatic sleep pressure. However, in these protocols there was a fixed phase relationship between the sleep homeostat and the circadian pacemaker, which prevented analysis of the interaction of the two processes. Measures of the homeostat and/or the pacemaker were also contaminated by masking effects (e.g., effects of uncontrolled motor activity, access to caffeine, and/or exposure to light except for constant routine studies). Finally, in some of those protocols, neurobehavioral assessments were given infrequently, resulting in temporal resolutions far too low to allow for estimation of circadian variation in performance.

High-frequency sleep/wake schedule studies, in which sleep/wake cycles were scheduled in a 1:2 time ratio with periods such as 20 min (e.g., Ref. 43), 90 min (e.g., Ref. 9), or 180 min (e.g., Ref. 49), have been used to demonstrate differences in sleep onset propensity and subjective alertness at different circadian phases, with the positive attribute that the protocols could be as short as a few days. However, most of these protocols had wake episodes that were too short to allow for accumulation of substantial homeostatic sleep pressure, lighting conditions that were uncontrolled, absent or marginal markers of circadian phase, cumulative sleep deprivation, and 28 h of enforced sleep deprivation before the experiment in most of the 20-min protocols.

Kleitman (36) first described a forced desynchrony protocol, which has subsequently been used by a number of investigators (12, 17, 18, 29, 32, 34, 42, 50). With the use of this protocol, subjects were studied in our laboratory on an imposed sleep/wake schedule with a 28-h period to force a desynchronization of the sleep/wake cycle from the endogenous circadian cycle (12, 17–19, 34, 42). Under these conditions, the endogenous circadian periods of temperature, melatonin, and cortisol averaged 24.18 h (12). With the imposition of the 28-h sleep/wake schedule, subjects were placed on a routine of bedtimes and waketimes that were 4 h later each sleep/wake cycle. Thus over the course of the 1-mo-long protocol, subjects were scheduled to bedtimes at many different circadian phases. Similarly, cognitive throughput and subjective alertness testing occurred at all possible ranges of circadian phase, with varying amounts of elapsed time since scheduled wake-

time (up to 18 h 40 min) occurring at each of these circadian phases. Data from this 28-h protocol demonstrated a nonadditive interaction of the two processes in the modulation of subjective alertness and cognitive throughput (19), wakefulness within sleep episodes (17, 20), and sleep structure, slow wave, and sleep spindle activity (18). Recent analyses of these data have shown a circadian modulation of low-frequency sleep spindles during non-rapid eye movement (NREM) sleep and of alpha frequency electroencephalogram (EEG) activity during rapid eye movement (REM) sleep (21).

The findings from the 28-h forced desynchrony protocol of circadian modulation of REM sleep and paradoxical timing of the sleep propensity rhythm were in good agreement with previous findings (as reviewed in Ref. 18). Other findings from this protocol, such as the near 24-h intrinsic period of the endogenous body temperature and melatonin rhythms and the nonadditive interaction of the circadian and homeostatic processes in the regulation of waking neurobehavioral function, were new. The close phase relationship between high plasma melatonin levels and optimal sleep consolidation remains a provisional finding.

On the basis of prior experience with a 28-h forced desynchrony protocol, which produced greater than normal sleep homeostatic drive due to its 18-h 40-min scheduled wake and 9-h 20-min scheduled sleep episodes, we constructed a comparison protocol with a 20-h forced rest/activity and sleep/wake cycle. Thus with only 13-h 20-min scheduled wake and 6-h 40-min scheduled sleep episodes, sleep homeostatic drive was lower than under normal (16-h wake and 8-h sleep) conditions. Also, rather than delaying the sleep episodes, subjects in the current protocol were required to undergo 4-h advances of their bedtimes and waketimes each rest/activity cycle. Blood samples were obtained each hour throughout the protocol for melatonin assay. Neurobehavioral functions were assessed using an entirely computerized assessment battery, which included additional testing of short-term memory, visual vigilance and reaction time, and another test of cognitive throughput.

## METHODS

### Subject Selection

Potential volunteers were recruited via poster, newspaper, and radio advertisement in the New England area. After passing a telephone screening interview, potential subjects gave informed consent and completed the following screening questionnaires: the Beck Depression Inventory, the Minnesota Multiphasic Personality Inventory-II, the Horne-Östberg Morningness-Eveningness Questionnaire, and a questionnaire covering sleep habits and physical health. Subjects showing no evidence of psychopathology or symptoms of a primary sleep disorder (e.g., insomnia, sleep apnea) on these screening instruments were scheduled for a physical examination, routine blood and urine chemistries, and a 12-lead electrocardiogram. Exclusion criteria included any probable or documented chronic or current major medical illness or injury. They also received a screening interview with a licensed clinical psychologist to rule out personal or familial history of major psychopathology and to determine their comprehension of and ability to comply with the investigational procedures. Finally, potential volunteers were interviewed by an investigator, and written informed consent was obtained for the protocol, which was approved by the Brigham and Women's Hospital's Human Research Committee.

Subjects were required to telephone the laboratory immediately prior to their scheduled bedtimes and immediately after their scheduled waketimes for at least 3 wk before the study to document adherence to an 8-h sleep episode, with bedtimes and waketimes fixed at their reported habitual times. All subjects completed at least 1 wk of wrist actigraphic monitoring (AMI-32, Ambulatory Monitoring, Ardsley, NY) of rest/activity patterns to verify compliance with the prestudy sleep schedule. Subjects were also instructed to abstain from intake of alcohol, caffeine, nicotine, over-the-counter or prescription medications, health food or vitamin supplements, or illicit substances for at least 2 wk before study. A urinary comprehensive toxicological analysis was conducted to verify that the subjects were drug-free on admission. Female subjects were given a blood pregnancy test both during screening and on admission to verify absence of pregnancy. Self-report data were collected on the timing of the menstrual cycle relative to admission date.

Data reported in this paper represent subjects found to have received placebo capsules only from an interim unblinding of our between-groups clinical trial of the efficacy of oral

Table 1. Subject demographics and estimated intrinsic period and amplitude of simultaneously estimated intrinsic and evoked components of core body temperature and plasma melatonin

Subjects			Core Body Temperature Data					Endogenous Melatonin Data		
Code	Age, yr	Gender	Study Duration, days	20-h Cycles Completed, no.	Intrinsic circadian period, h:min	Endogenous amplitude, °C	Evoked amplitude, °C	Intrinsic circadian period, h:min	Endogenous amplitude, pmol/l	Evoked amplitude, pmol/l
<i>a</i>	25	M	27	24	24:15	0.24	0.13	24:15	97.7	25.9
<i>b</i>	25	M	27	24	23:58	0.21	0.25	24:01	249.4	25.0
<i>c</i>	24	M	18	17	*	0.24	0.17	24:00	43.6	7.0
<i>d</i>	19	M	17	15	*	0.20	0.10	24:02	151.1	38.5
<i>e</i>	19	M	27	24	24:17	0.27	0.36	24:15	196.6	47.7
<i>f</i>	27	F	27	24	24:10	0.21	0.18	24:09	123.3	15.3
Mean	23.2	—	—	—	24:10	0.23	0.20	24:07	143.6	26.6
SD					0:09	0.03	0.09	0:07	72.9	14.8

\* Study duration insufficient to allow precise estimation of intrinsic circadian period from temperature data. M, male; F, female.

melatonin administration as a hypnotic (see Table 1 for subject demographics).

### Study Admission

Six subjects, one female and five male (age 19–27 yr, mean 23.2 yr), were studied in the Environmental Scheduling Facility or the Intensive Physiological Monitoring Unit of the General Clinical Research Center at Brigham and Women's Hospital. They were oriented to the study procedures and systems in their suite. They received ~3 h of training on the computerized neurobehavioral assessment battery, including feedback from an investigator on their performance during the first three baseline wake episodes. For all performance tasks, subjects were instructed to perform as quickly as possible, but without sacrificing accuracy.

### Sleep/Wake and Light/Dark Cycle

In this 27-day protocol, subjects were scheduled to an initial baseline segment consisting of three wake/sleep and light/dark repetitions of 24-h cycles with 16-h wake episodes and 8-h sleep episodes. The sleep schedule during the baseline segment was based on the bedtimes and waketimes reported by the subject during the prestudy screening week immediately before admission. Subjects arrived at the laboratory in the middle of the afternoon during that first 16-h wake episode. Following three baseline cycles, they were scheduled to 24 repetitions of a 20-h cycle, with 13-h 20-min wake episodes and 6-h 40-min sleep episodes. After this forced desynchrony segment, subjects were again scheduled to the 24-h cycle for three repetitions for a postdesynchrony baseline segment (see Fig. 1). The forced desynchrony segment was thus similar to traveling eastward four time zones per day, with bedtimes and waketimes advanced by 4 h per cycle. Ambient lighting in the subject's living area and bathroom was verified to be ~15 lx (angle of gaze) during wake episodes and <0.03 lx during sleep episodes. We selected a relatively dim lighting level during scheduled wake to minimize both the possibility of relative coordination of circadian pacemaker to the light/dark cycle (37) and the direct arousing effect seen with brighter light (as reviewed in Ref. 8). From admission to end of study, subjects were kept in an environment free of time cues, with no access to timepieces of any type or any information about time or calendar day from the nursing or technical staff or from the investigators. Two subjects were disempaneled midway through the protocol for minor illnesses. Their data are included in this report, because they completed sufficient 20-h cycles to allow for analysis of endogenous circadian phase and period (*subject c* was disempaneled due to tooth pain, after completing 17 20-h cycles; *subject d* was disempaneled due to forearm dermal irritation related to intravenous catheter use after completing 15 20-h cycles).

### Circadian Parameters

Core body temperature was recorded continuously from a rectal temperature thermistor (Yellow Springs Instrument Company, Yellow Springs, OH), with data stored in 1-min epochs. Hourly blood plasma samples were obtained from an indwelling catheter in a forearm vein for analysis of melatonin. Immediately after collection, each whole blood sample was placed in a Vacutainer tube with EDTA, centrifuged at 2°C for 10 min at 2,200–2,800 rpm, and the separated plasma was placed in an aliquot tube and frozen at –25°C. Plasma samples were assayed by radioimmunoassay for melatonin (assay sensitivity 10.1 pmol/l; DiagnosTech, Osceola, WI).

Separate nonorthogonal spectral analyses of the core body temperature and plasma melatonin data collected during the

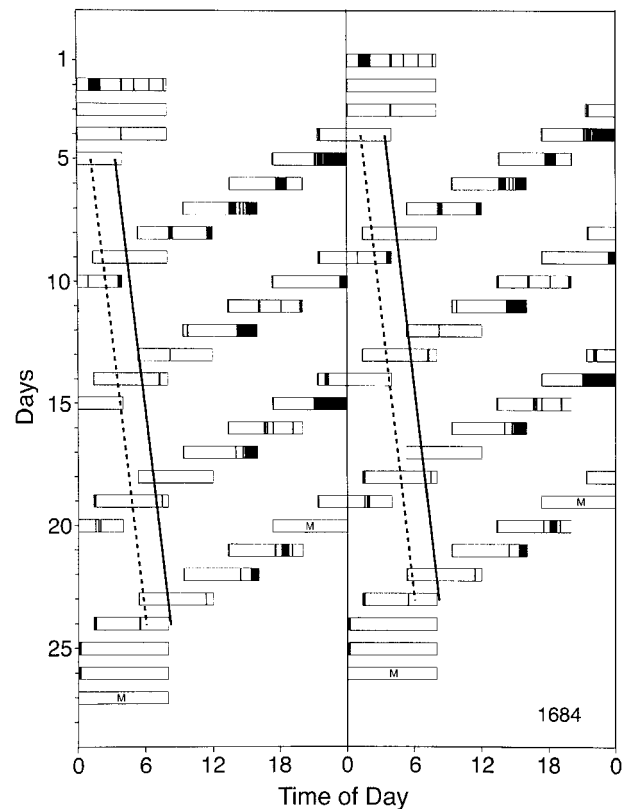


Fig. 1. Double-raster plot of 27-day forced desynchrony protocol. Each successive 24-h period is plotted to *right* and below preceding 24-h period. In this example from 1 subject (*ID 1684*), habitual bedtime was 2400 and habitual waketime was 0800. After 3 baseline cycles of 24 h, subject was placed on a 20-h sleep/wake and light/dark cycle (sleep episodes indicated with horizontal boxes). All 5-min epochs of the polysomnogram comprised of  $\geq 50\%$  wakefulness are also shown (vertical bars within horizontal boxes indicating sleep episodes). Two sleep episodes for which complete data were not available are indicated with an "M." Fitted peak of endogenous plasma melatonin levels (dashed line) and trough of core body temperature (solid line) drift to a later phase position relative to clock time in this subject, both indicating a circadian period of 24 h 15 min.

forced desynchrony portion of the protocol were used to simultaneously estimate the evoked effect of the sleep/wake cycle and the modulation from intrinsic circadian oscillation (12). For both the core body temperature and plasma melatonin data, the regression model was fit with one fundamental and one additional harmonic for the intrinsic circadian component and a fundamental and seven additional harmonics for the evoked component. For core body temperature only, the model included a parameter for serially correlated noise. Estimations for the intrinsic amplitude of the temperature and plasma melatonin rhythms were derived from composite measures of a fundamental component plus one harmonic. Estimations of evoked amplitude in these measures were derived from composite measures of a fundamental component plus seven additional harmonics.

In addition to yielding an estimation of intrinsic period, the regression analysis also yielded an estimated clock time for the first core body temperature nadir. Thus each minute of an individual subject's forced desynchrony protocol could be assigned a circadian phase varying from 0 to 360 degrees, based on the offset from the first core body temperature minimum (circadian phase 0) and the circadian period estimate given by the regression analysis (47).



For each subject, plasma melatonin data were folded at the circadian periods of the core body temperature data. They were then analyzed with a bin width of 40 degrees for the circadian component ( $\sim 2.67$  h) and a bin width of 30 degrees for the sleep-wake dependent component ( $\sim 1.67$  h) referenced to the circadian phase and period data from the body temperature, as outlined above.

### *Sleep Recordings*

Referential electrooculogram (EOG) and scalp EEG, and chin electromyogram were acquired during all sleep episodes with a caplike sensor array (Sleep\*Net, Physiometrix, North Billerica, MA). Sleep data were collected using the Nicolet UltraSom (Nicolet Biomedical, Madison, WI) or the Vitaport 2 Digital Sleep Recorder (Temec Instruments, Kerkrade, Netherlands). Data were acquired on both systems via amplification and analog-to-digital conversion (low-pass filter = 35 Hz and high-pass filter = 0.3 Hz for UltraSom and low-pass filter = 70 Hz and high-pass time constant = 0.33 s for Vitaport 2). Sleep data were scored in 30-s epochs in accordance with standard criteria (41) by a polysomnographic technician or an investigator, each with  $>7$  years of experience with polysomnography. Each 30-s epoch of the sleep recordings was assigned a circadian phase based on the core body temperature data (as described above) and an elapsed time-into-sleep episode. Sleep latency, defined as the elapsed time from lights out to the first epoch of any sleep stage and latency from sleep onset to REM sleep were log transformed due to the skewed distributions of raw scores.

### *Neurobehavioral Assessment*

At 2-h intervals beginning 2 h after scheduled awakening, subjects were administered a 30-min computerized neurobehavioral assessment battery consisting of short-term memory [Probed Recall Memory test (PRM); Ref. 22], simple reaction time and visual vigilance [Psychomotor Vigilance Task (PVT); Ref. 23], and two cognitive throughput tasks [Addition/Calculation test (ADD); Refs. 19 and 34, Digit Symbol Substitution Test (DSST)]. At 30-min intervals beginning 30 min after scheduled awakening, subjects were administered computerized tests of alertness/sleepiness [Karolinska Sleepiness Scale (KSS); Ref. 26] and mood. Waking EEG and EOG were recorded from 3 h after scheduled waketime until 1 h before lights out each wake episode to document that wakefulness was maintained in each scheduled wake episode. Technicians awakened subjects whenever they noted that the EEG indicated the subject had fallen asleep.

### *Data Reduction and Statistical Procedures*

In all results reported below, individual data points were assigned a circadian phase and either 1) an elapsed time into the wake episode (neurobehavioral measures); 2) an elapsed time into the sleep episode (polysomnographic data); or 3) an elapsed time into the 20-h cycle measured from scheduled waketime (endogenous plasma melatonin data). Mean performance scores during the entire forced desynchrony segment of the protocol were used to calculate deviation-from-mean scores for all neurobehavioral measures collected during forced desynchrony. Data reduction was accomplished by averaging, within subjects and within measures, all data points assigned the same circadian phase and elapsed time. Thus each subject contributed equally to subsequent analyses. For analysis of circadian modulation of sleep structure, a bin width of 60 degrees ( $\sim 4$  h) was selected. Neurobehavioral data were analyzed with bin widths of 40 degrees for the circadian component ( $\sim 2.67$  h) and 40 degrees for the wake-

dependent component ( $\sim 2.22$  h). Due to more frequent administration, the KSS data were analyzed with 30-degree bins for both components.

Repeated-measures analyses of variance procedures were used with the SAS software for Windows (version 6.12 for PC, SAS Institute, Cary, NC). All  $P$  values are reported with Huynh-Feldt correction for sphericity, but the original degrees of freedom are reported.

## RESULTS

### *Intrinsic Period of Temperature and Melatonin*

The intrinsic circadian periods estimated from the core body temperature and plasma melatonin data were on average slightly longer than 24 h, although subjects were observed to have intrinsic circadian periods both shorter and longer than 24 h (see Table 1). Thus, in the 20-h forced desynchrony protocol, the circadian pacemaker oscillated at a close to 24-h period and desynchronized from the scheduled sleep/wake and light/dark cycle period of 20 h.

### *Endogenous Plasma Melatonin*

There was a significant effect of elapsed time into the 20-h day [ $F(8,24) = 9.35, P < 0.0004$ ]. There was also a significant effect of circadian temperature phase [ $F(8,24) = 23.80, P < 0.0058$ ]. The interaction between elapsed cycle time and circadian phase was also significant [ $F(84,192) = 2.84, P < 0.0372$ ]. Melatonin levels declined during elapsed hours of the wake episode, rising coincident with the onset of the sleep episode (Fig. 2, right). Average melatonin levels reached a peak shortly before the core body temperature minimum (Fig. 2, left). The melatonin data suggest greater circadian than activity/rest cycle modulation of this hormone (Fig. 2).

### *Sleep Structure*

In separate repeated-measures analyses of variance, there were significant effects of circadian phase at the beginning of the sleep episode on sleep latency [ $F(5,25) = 4.28, P = 0.0066$ ] and latency from sleep onset to REM sleep [ $F(5,25) = 3.06, P = 0.0341$ ]. Sleep latency and REM sleep latency were shortest near or shortly after 0 degrees and longest at 240 degrees of core body temperature (see Fig. 2).

With regard to sleep continuity, there was a significant effect of circadian phase on sleep efficiency [ $F(5,25) = 5.45, P = 0.0016$ ], minutes of total sleep time [ $F(5,25) = 5.69, P = 0.0012$ ], and minutes of wake after sleep onset [ $F(5,25) = 5.58, P = 0.0023$ ]. In all cases, sleep continuity was highest in sleep episodes centered at 0 degrees and lowest at 240 degrees (see Fig. 2). Evidence of the effect of circadian phase of the sleep episodes on sleep continuity can be seen in a double raster plot of each sleep episode from a single subject (Fig. 1). It is evident that in sleep episodes centered around 240 degrees, significant sleep disruption occurs, particularly in the last third of the sleep episodes.

For a more detailed inspection of sleep architecture, data were assigned both a circadian phase relative to the middle of the sleep episode and divided by third of the 6-h 40-min sleep episode (see Table 2). With

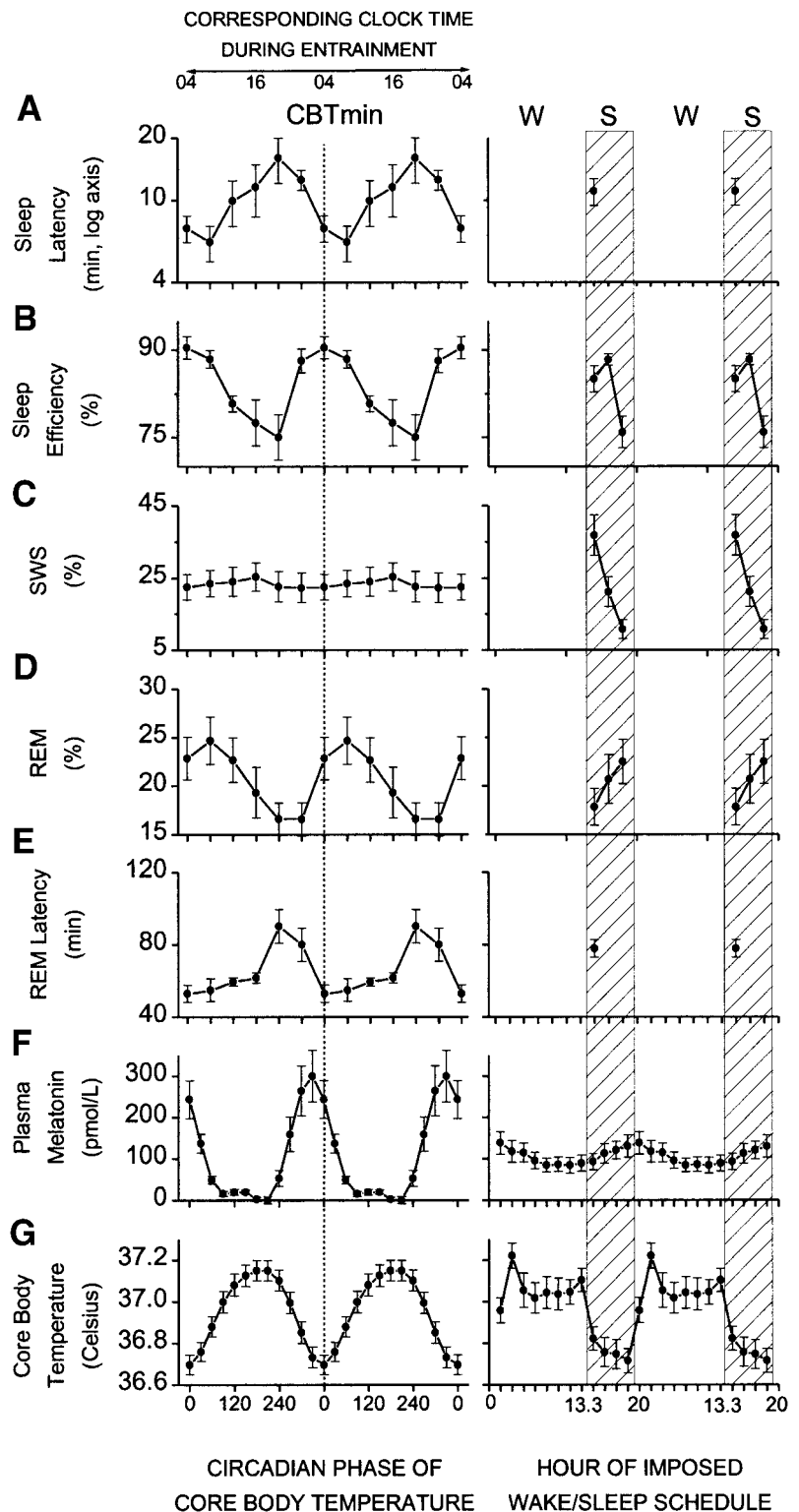


Fig. 2. Double plots of body temperature, endogenous plasma melatonin, and sleep parameters. Except for *G*, all data in *left* panels are plotted with data folded at endogenous circadian period as determined by core body temperature for each subject. Data in all *right* panels are folded at forced 20-h sleep-wake cycle. All data were averaged first within each subject across all sleep episodes (sleep data) and sleep/wake cycles (melatonin and temperature data), then averaged across subjects, with plotted points reflecting mean values and their respective SE. Sleep latency is expressed in minutes, plotted on a logarithmic scale (*A*). Sleep efficiency is expressed as a percentage of time in bed (*B*). Slow-wave sleep (SWS; *C*) and rapid eye movement (REM) sleep (*D*) are expressed as a percentage of total sleep time. REM latency (*E*) was expressed as minutes from sleep onset to first epoch of REM sleep. Melatonin data are depicted with raw plasma levels folded either at endogenous circadian period as determined by temperature (*F*, *left*) or at forced 20-h sleep-wake cycle (*F*, *right*). Core body temperature was also determined by simultaneously estimated intrinsic circadian variation (*G*, *left*) and evoked effect of sleep/wake cycle (*G*, *right*). W, wake; S, sleep.

regard to sleep continuity, there were significant main effects of both circadian phase and third of the sleep episode, as well as significant two-way interactions for the variables of sleep efficiency, minutes of total sleep time, and percent of epochs scored as wakefulness. The degree of circadian modulation of sleep continuity increased in the last third of the sleep episodes, show-

ing maximal circadian drive for sleep and minimal intrusion of wakefulness in sleep episodes centered at 0 degrees (Fig. 3). Sleep continuity was also lowest during the last third of the sleep episodes. Similarly, the lowest percentage of stage 1 NREM sleep was seen in the first third of the sleep episodes or, during the later parts of the night, near the circadian temperature

**Table 2. Analysis of sleep architecture by third of sleep episode and circadian phase**

Variable	Third of Sleep Episode		Circadian Phase		Interaction	
	<i>F</i> (2,10)	<i>P</i>	<i>F</i> (5,25)	<i>P</i>	<i>F</i> (10,50)	<i>P</i>
TST, min	10.63	0.0071	6.61	0.0005	2.76	0.0191
SE, %	10.52	0.0072	6.56	0.0005	2.76	0.0191
Wake, %	10.47	0.0073	6.66	0.0005	2.78	0.0187
Stage 1, %	13.04	0.0016	3.12	0.0383	1.69	NS
Stage 2, %	17.41	0.0006	4.12	0.0508	1.56	NS
SWS, %	29.15	<0.0001	0.70	NS	0.81	NS
REM, %	2.94	0.1425	9.86	0.0002	4.28	0.0003

TST, total sleep time; SE, sleep efficiency; SWS, slow-wave sleep; REM, rapid eye movement; NS, not significant.

nadir. Percentage of stage 2 NREM sleep increased across the sleep episodes, concurrent with a decrease in slow-wave sleep (SWS). However, of the two stages, only stage 2 sleep showed a significant circadian modulation as well. Finally, REM sleep showed a significant effect of circadian phase and trend for third of the sleep episode, and a significant interaction of the two effects. The highest percentage of time spent in REM sleep was seen just after the circadian temperature nadir.

#### Waking Neurobehavioral Assessment

For the ADD task, there were significant main effects of elapsed wake and circadian phase. For the DSST, there was a trend in the main effect of elapsed wake and a significant effect of circadian phase. For the PRM task, there was a significant main effect of elapsed wake, but not a significant effect of circadian phase. For

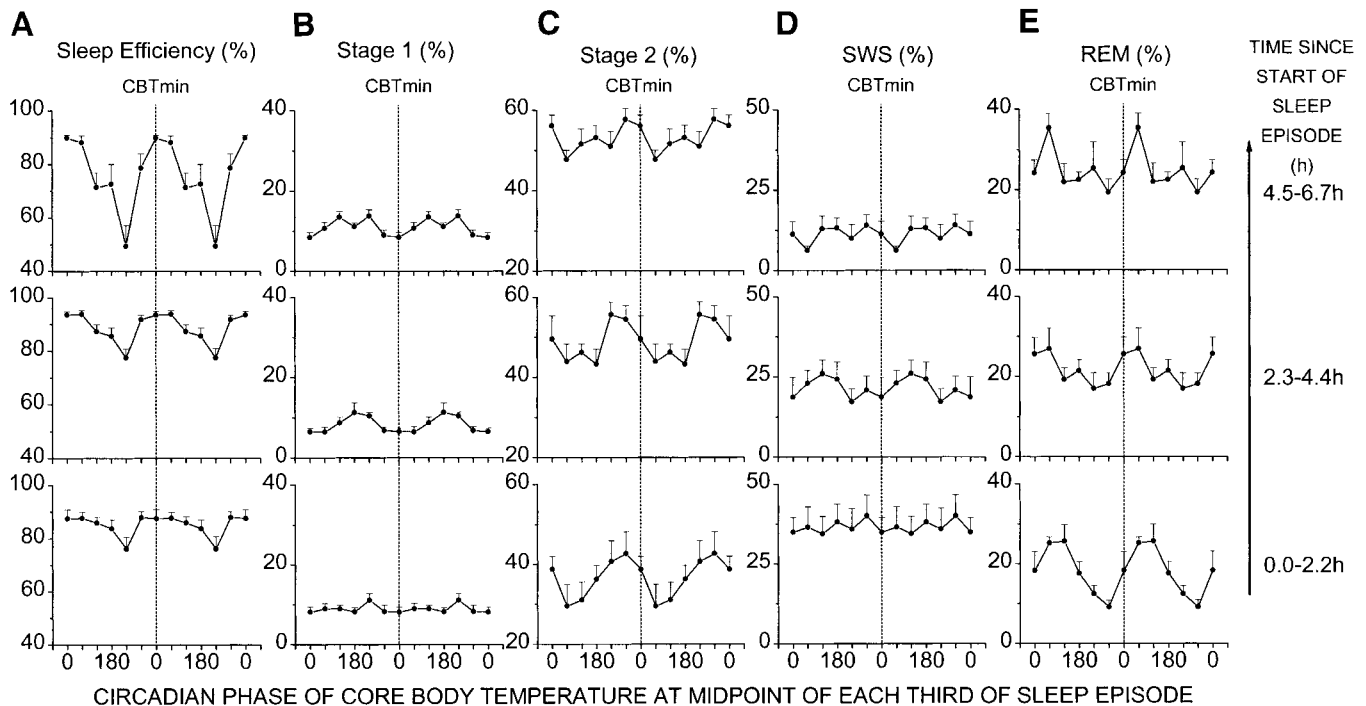
the PVT, there were significant main effects of elapsed wake for median reaction time and lapses (lapses defined as reaction times  $\geq 500$  ms) and circadian phase. In none of these tasks were there significant interactions of elapsed wake and circadian phase. However, for all tasks, performance worsened with increased hours of wakefulness and near the circadian temperature nadir. During the first 3 h 20 min of wakefulness, performance across the test battery remained better than the mean level from the entire forced desynchrony portion of the protocol. Also within this time bin, there was little circadian variation evident in the tasks, except for the ADD task. In marked contrast, during the last bin of elapsed wakefulness (from 10 h to 13 h 20 min), performance levels were worse than the mean level. Also, with increased hours of elapsed wakefulness, and hence increased sleep homeostatic pressure, the degree of circadian modulation became greater, although not reaching statistical significance (see Figs. 4 and 5 and Table 3).

With regard to subjective sleepiness/alertness, there were significant main effects of elapsed wake and circadian phase. There was also a significant interaction of the two factors; the degree of circadian modulation increased with hours of elapsed wakefulness (Fig. 5).

#### DISCUSSION

##### General Findings

In this forced desynchrony protocol with 20-h light/dark and activity/rest cycles, we found that the intrinsic



**Fig. 3.** Depiction of interaction of time into each sleep episode (rows) and circadian phase (abscissa of each plot, determined as circadian phase position of each 30-s epoch of sleep relative to core body temperature minimum) on sleep efficiency and architecture. Plotted points show mean values and their respective SE. Sleep efficiency (A) is expressed as a percentage of time in bed. Sleep stages (B-E) are expressed as a percentage of total sleep time. CBTmin, core body temperature minimum.

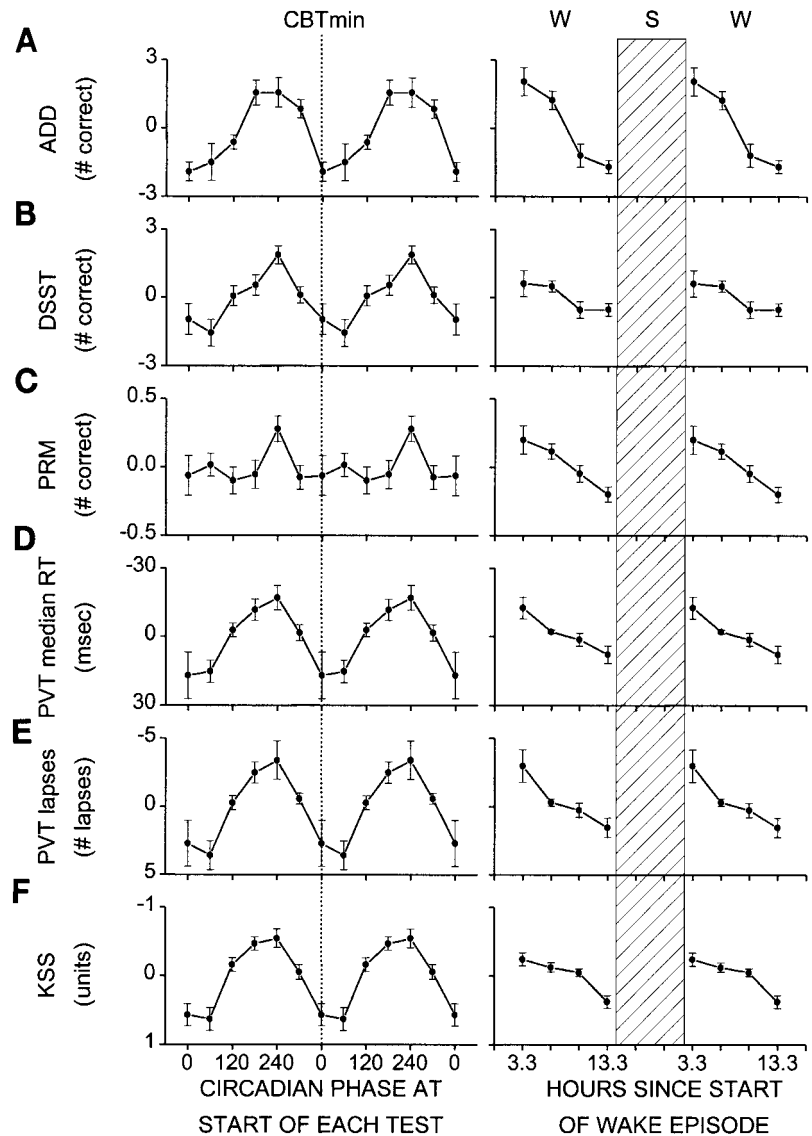


Fig. 4. Double plots of main effects of circadian phase relative to minimum of core body temperature (*left*) and duration of prior scheduled wakefulness (*right*) on neurobehavioral measures. Plotted points show deviation from mean values during forced desynchrony section of protocol and their respective SE. For all panels, values plotted lower in panel represent impairment on that neurobehavioral measure. Addition/Calculation test (ADD; *A*), Digit Symbol Substitution Test (DSST; *B*), and Probed Recall Memory test (PRM; *C*) scores are derived from total number of correct responses. Psychomotor Vigilance Task (PVT) results represent median reaction time (*D*) and total number of lapses (*E*, reaction times >500 ms). Karolinska Sleepiness Scale (KSS) scores (*F*) represent responses on this 1–9 Likert-type scale.

sic period of the circadian pacemaker averaged 24.1–24.2 h. Also, even with only 13 h 20 min of scheduled wakefulness preceding each sleep episode, there was still significant homeostatic and circadian modulation of sleep structure, with the highest sleep efficiency occurring in sleep episodes bracketing the melatonin maximum and core body temperature minimum. However, at these same circadian phases and toward the end of each wake episode, maximal impairment of neurobehavioral functioning was observed across all subjective and objective measures. The results make clear the importance of the sleep/wake homeostatic system, the endogenous circadian system, and their interaction in the modulation of sleep and neurobehavioral functions in healthy young adults.

#### *Intrinsic Period: Melatonin and Core Body Temperature*

In this study, we found that under a forced desynchrony protocol with a 20-h light/dark and activity/rest cycle, the intrinsic period of the circadian pacemaker as

derived from core temperature and plasma melatonin averaged much closer to 24 h than earlier reported in a variety of studies (e.g., Refs. 2, 35, 50), but consistent with more recent estimations (e.g., Refs. 7, 10, 12, 29, 39). The period estimations appeared quite stable in the subjects who completed the entire protocol, and were nearly identical when estimated based on either continuous recordings of core body temperature or hourly plasma melatonin samples. Importantly, the variability of our estimations of intrinsic circadian period was quite small both within and across subjects, in contrast to the report of a very short duration (5 day) 20-h forced desynchrony protocol (29), which found higher intra- and intersubject variability in estimations of period. In addition, our estimations of intrinsic period were not confounded by exposure to potential nonphotic synchronizers (e.g., access to time information, direct contact with other subjects, activity recurring at the same circadian phase) and potential interactions between self-selected sleeping times and melatonin secretion (39).



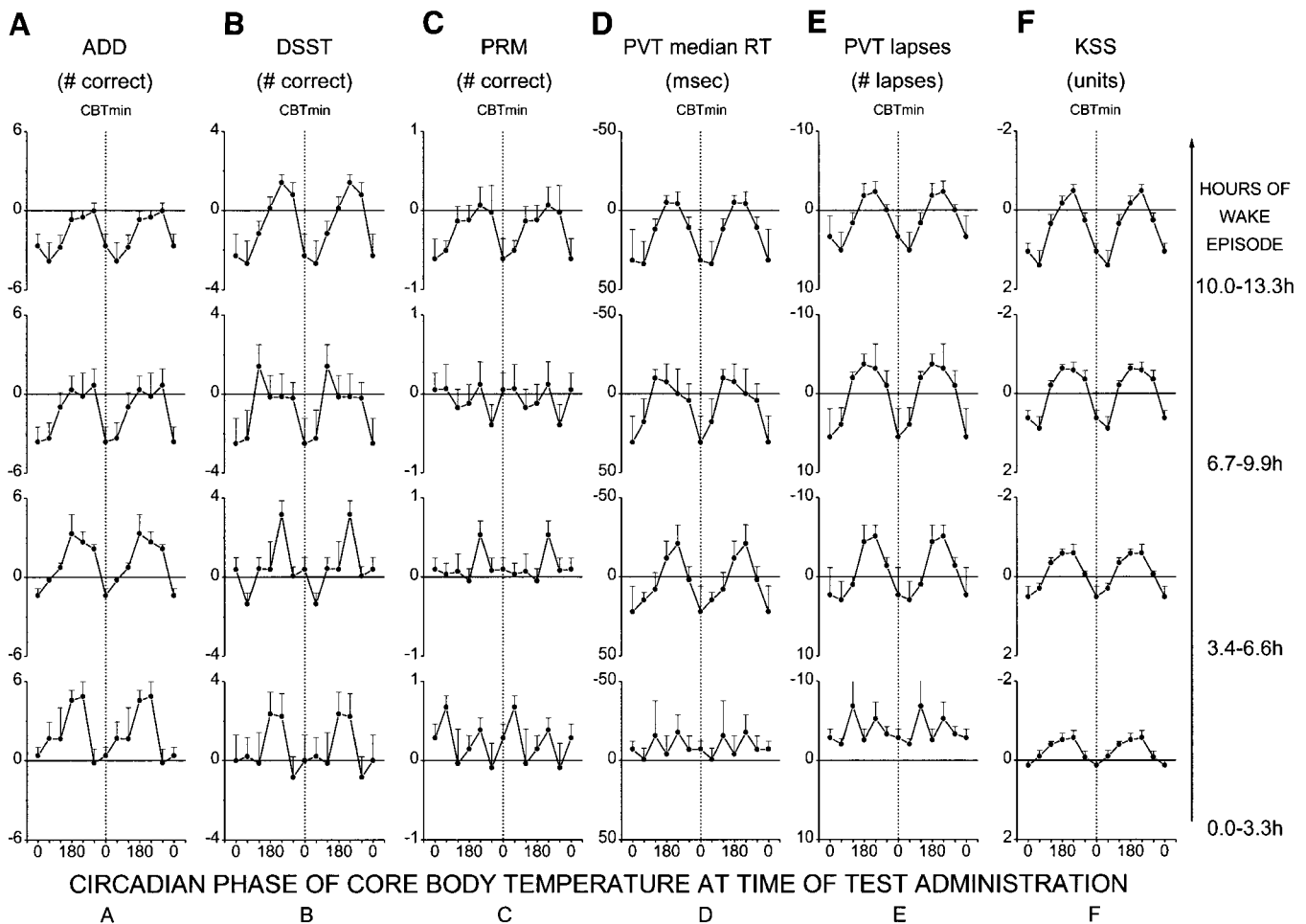


Fig. 5. Depiction of interaction of duration of prior scheduled wakefulness (rows) and circadian phase (abscissa of each plot, determined as circadian phase position of time of each individual test administration relative to core body temperature minimum) on neurobehavioral measures. Plotted points show deviation from mean values during forced desynchrony section of protocol and their respective SE. For all panels, values plotted lower in panel represent impairment on that neurobehavioral measure. As in Fig. 4, ADD (A), DSST (B), and PRM (C) scores are derived from total number of correct responses. PVT results represent median reaction time (RT; D) and total number of lapses (E, reaction times >500 ms). KSS scores (F) represent responses on this 1–9 Likert-type scale.

Furthermore, we found a stable phase relationship between melatonin and core body temperature, with the onset of melatonin secretion and the initial decline in core body temperature occurring at a circadian phase normally encountered a few hours before habitual

bedtime. Peak melatonin levels were reached just before the core body temperature minimum at a circadian phase value normally encountered a few hours before the habitual waketime.

Table 3. Analysis of neurobehavioral measures by elapsed time into wake episode and circadian phase

Variable	Duration of Prior Scheduled Wakefulness		Circadian Phase		Interaction	
	F(3,15)	P	F(5,25)	P	F(15,75)	P
ADD	12.07	0.0003	5.23	0.0051	1.45	NS
DSST	2.79	0.0910	3.51	0.0220	1.33	NS
PRM	5.77	0.0079	1.82	NS	0.84	NS
PVT: median RT	5.21	0.0553	4.60	0.0545	1.27	NS
PVT: lapses	9.94	0.0066	8.59	0.0013	1.51	NS
KSS	4.94	0.0045	12.20	0.0002	1.82	0.0590

ADD, Addition/Calculation test; DSST, Digit Symbol Substitution Test; PRM, Probed Recall Memory test; PVT, Psychomotor Vigilance Task; KSS, Karolinska Sleepiness Scale.

#### Homeostatic and Circadian Modulation of Sleep Propensity and Sleep Structure

**Sleep initiation and continuity.** In the analyses of the sleep episodes as a whole, the sleep latency data point to the temperature nadir (0 circadian degrees) as a time of maximal circadian drive for sleep and approximately 240 degrees as a time of maximal circadian drive for wakefulness or minimal circadian drive for sleep. These data are consistent with the earlier observations from high-frequency sleep/wake schedules (9, 38, 49), free-running studies (13, 44, 47, 53), and 28-h forced desynchrony studies (17, 18). However, it was somewhat surprising that even with phase advances of the sleep/wake episodes that placed scheduled bedtime near what has been called the “forbidden zone for sleep” (38)



or the “wake maintenance zone” (45), we did not observe pathologically long sleep latencies. These data suggest that with at least 13 h 20 min of wakefulness preceding each sleep attempt, healthy, young normal-sleeping individuals do not exhibit sleep-onset insomnia in this protocol, even with sleep scheduled at a phase corresponding to late in the habitual waking day. The word “forbidden” is thus perhaps a bit too strong. In fact, in the ultrashort sleep studies, subjects were given only 7 min to fall asleep in each attempt. Our data indicate that at the circadian phase associated with the wake maintenance zone (45), sleep latencies were at their peak, averaging nearly 20 min. Hence, our data are consistent with those of Lavie (38) and with the concept of a wake maintenance zone. Psychophysiological insomniacs studied on this protocol might exhibit an even more impressive variability of sleep latencies when bedtimes are varied over the range of the circadian cycle.

We also observed significant effects of circadian phase on several measures of sleep continuity: minutes of total sleep time, sleep efficiency, and percentage of wakefulness. Across these highly related measures, sleep episodes centered at the phase of 0 circadian degrees had the highest sleep continuity and the lowest percentage of wakefulness. Conversely, sleep episodes centered around the 240 degree circadian temperature phase had the lowest sleep continuity and the greatest amount of wakefulness. These data are also consistent with findings from both free-running studies (14, 53) and 28-h forced desynchrony protocols (17, 18). The data are also consistent with the observations that the duration and termination of self-selected sleep episodes in free-running subjects were highly related to the phase of the core body temperature rhythm, such that subjects tended to wake up on the rising phase of the temperature rhythm (13, 53). Taken together, the sleep initiation and continuity data suggest that in normal healthy adults without sleep complaints, the greatest sleep propensity and hence the most efficient, consolidated sleep would be obtained under conditions seen in normal entrainment, with the habitual bed- and wake-times bracketing the endogenous circadian temperature nadir and melatonin maximum.

*Sleep architecture.* The analyses of sleep episodes by third of sleep episode and by midsleep circadian phase address the combined effects of the sleep/wake homeostat and the circadian pacemaker on sleep continuity and structure. Sleep fragmentation was worst during the last third of the sleep episode, indicating that with low homeostatic pressure for sleep, circadian modulation of sleep fragmentation becomes impressively evident. This is consistent with our findings from the 28-h forced desynchrony protocol (18). Also, as described elsewhere (16, 18), we observed a sleep-dependent disinhibition of REM sleep. In addition to showing profound circadian modulation of REM percentage and latency from sleep onset to REM sleep, as has been shown in free-running studies (13, 14), there was suggestive evidence of a trend increase in REM sleep percentage as homeostatic pressure for sleep abated.

Finally, we observed a dominant homeostatic modulation of SWS, with the amount of SWS declining across successive thirds of the individual sleep episode regardless of circadian phase position of the sleep episode, as has also been shown in free-running studies and forced desynchrony protocols (18, 48). Thus these analyses confirm that SWS is a simple but informative indicator of both overall homeostatic sleep pressure built up before sleep and the satiation of homeostatic sleep pressure brought about by continued time spent sleeping, as proposed by Webb and Agnew (46).

#### *Temporal Relation of Melatonin, Core Body Temperature, and Sleep*

The close correspondence between the phase of the rhythms of core body temperature, endogenous plasma melatonin, and sleep propensity was consistent with our previous findings (18, 21). With regard to possible effects of body temperature on sleep, melatonin has been reported to be a hypothermic agent when given exogenously (25, 31), but it remains unclear whether melatonin levels in the true physiological range produce significant declines in body temperature (reviewed in Ref. 6). We found a small but significant evoked effect of the imposed 20-h routine on the plasma melatonin levels consistent with our report from the 28-h day (42). Whether this effect is evoked by the timing of the sleep/wake cycle or the associated changes in lighting (although very dim throughout the protocol during wake) or posture cannot be determined from this protocol. In general, it remains unclear the extent to which melatonin and core body temperature may have been causally related in this protocol and the extent to which melatonin and/or core body temperature may have been causally related to sleep propensity.

#### *Homeostatic and Circadian Modulation of Neurobehavioral Functions*

This protocol used a broader range of neurobehavioral measures than has previously been reported in studies examining both sleep/wake homeostatic and circadian modulation of alertness and cognitive performance. Across all measures, we observed homeostatic modulation of performance, with impairments in subjective sleepiness and cognitive performance increasing with duration of elapsed wakefulness. This is impressive, given that the scheduled wake episodes were several hours shorter than the duration of a habitual waking day, and hence there was less time for homeostatic sleep pressure to build.

In addition, we noted significant circadian modulation of these measures. Across all measures, neurobehavioral functioning was best at ~240 circadian degrees and showed maximal impairment at or just after 0 circadian degrees. In terms of the physiology, maximal circadian promotion of neurobehavioral functioning was seen near the core body temperature maximum and just before the release of melatonin, and maximal circadian impairment was seen just after the nadir of core body temperature and the maximum of melatonin

secretion. Under conditions of entrainment, this maximal circadian drive for wakefulness would occur near the end of the habitual waking day. Similarly, maximal circadian drive for sleep and, hence, maximal impairment of waking neurobehavioral functioning, would occur near the end of the habitual sleep episode. All of these findings are consistent with the subjective alertness and cognitive throughput results reported from the 28-h forced desynchrony protocol (19).

Only the subjective alertness measure showed statistically significant interaction between the homeostatic and circadian processes. However, visual inspection of the data (Fig. 5) suggested interesting potential differences between the tasks. For example, it appeared that there was circadian modulation of the two cognitive throughput tasks regardless of the level of homeostatic sleep pressure. These two tasks have heavy attentional and short-term memory loading, which may make them more sensitive to circadian modulation than the other tasks. For the PRM task and the two indexes of the PVT, the amplitude of circadian modulation appeared to increase with increasing hours of wakefulness. Furthermore, studies on a larger data set will be required to determine whether these intriguing inter-task differences in the extent of circadian-homeostatic interaction are upheld.

A possible explanation for the failure to find a significant interaction between the homeostatic and circadian factors in these analyses lies in the fact that the absolute level of homeostatic pressure was never very great in this protocol; none of the subjects reported great difficulty remaining awake during the wake episodes, and the duration of enforced wakefulness was limited to 13 h 20 min. In other reports of a nonlinear interaction of the pacemaker and the homeostat in the modulation of waking neurobehavioral performance (19, 52), wake episodes were scheduled to between 18 h 40 min and 28 h 34 min. Thus it may not have been possible to find significant interactions in the present protocol due to an insufficient physiological challenge to the sleep homeostatic system. Finally, limited statistical power associated with this small sample size may have contributed to the lack of significance in these interactions, except as demonstrated for subjective alertness.

### *Implications*

In this 20-h forced desynchrony protocol, we found patterns of sleep and neurobehavioral regulation similar to those found in a 28-h forced desynchrony protocol (19). In both the sleep and neurobehavioral data, we found further evidence to support the conclusion that the endogenous circadian pacemaker actively promotes both sleep initiation and sleep consolidation, the latter being particularly critical when homeostatic pressure for sleep is nearly satiated toward the end of a sleep episode. In contrast, during wake episodes scheduled to occur during the normal circadian time of wakefulness (60–240 degrees), the pacemaker has an active role in the promotion of wakefulness, which is particularly useful in offsetting the decline in alertness and perfor-

mance resulting from homeostatic sleep pressure shown with increased hours of wakefulness (11). If not for these active modulations from the circadian pacemaker, sleep would become very fragmented after the first few hours and it would be difficult to maintain optimal alertness during even a normal, 16-h duration of desired wake. Taken together, when an optimal relationship exists between the sleep/wake schedule and the phase of the endogenous circadian system, the modulatory influences of the sleep/wake homeostat and the pacemaker combine in this complementary manner to allow for a relatively long (7–9 h) consolidated sleep episode and a stable level of waking neurobehavioral function across the habitual waking day (15–17 h; Refs. 11, 17). Although these findings are clear in this population of healthy young adults, it could be the case that differences may be found between these young adults and older adults (20) or adults with sleep complaints.

In addition to providing additional information about the basic, yet critically important, interaction of the sleep/wake homeostatic and the endogenous circadian processes, data from the forced desynchrony protocol allow us to make testable predictions for sleep and waking functioning in individuals with sleep/wake schedules other than the traditional 8:16-h pattern. In jobs requiring high levels of attention and performance over extended durations of time (e.g., long-haul aircrews or truck drivers, medical house staff, nuclear power plant operators), medical advisers or supervisors using computerized models that include this nonlinear interaction of the sleep homeostat and the circadian pacemaker could warn workers of times of performance vulnerability and inadvertent sleep onset, as well as advise optimal timing of sleep or other countermeasures against neurobehavioral deficit (see Ref. 40). The forced desynchrony protocol itself also provides a singular opportunity to quantify the homeostatic- and circadian-dependent properties of various medications, including stimulants and hypnotics, medications with a desired time of action (e.g., antihypertensives), and medications with variable side-effect profiles (e.g., anti-cancer medications). Additionally, awareness of the relationship between the sleep homeostat and the endogenous circadian pacemaker may increase our understanding of the most prevalent sleep disorder, insomnia.

In summary, we found that healthy young subjects on a 20-h forced desynchrony protocol exhibited significant modulation of sleep structure and waking neurobehavioral functions from the sleep/wake homeostatic and endogenous circadian systems. We saw progressive impairment on multiple neurobehavioral tasks with increasing duration of prior wakefulness, suggesting the powerful impact of accumulating homeostatic sleep pressure even with scheduled wake episodes several hours shorter than the habitual 16- to 17-h duration. Relative to clock hours observed during conditions of normal entrainment and given the average intrinsic circadian period of 24.1–24.2 h found in this protocol, the timing of maximal circadian drive for wakefulness

occurred just before the habitual bedtime, and the maximal drive for sleep occurred just before habitual waketime. These data support the concept that under normal conditions, the paradoxical interaction of the homeostatic and circadian systems serves to consolidate both sleep and wakefulness in healthy young adults.

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