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A biphasic daily pattern of Slow Wave Activity during a two-day 90-minute sleep wake schedule

W.C. DUNCAN[1], G. BARBATO[1,2], I. FAGIOLI[3†],
D. GARCIA-BORREGUERO[4], T.A. WEHR[1]

[1] *Mood and Anxiety Disorders Program, IRP, NIMH, Bethesda (MD), USA;*
[2] *Department of Psychology, Second University of Naples, Italy;*
[3] *Department of Psychology, University of Florence, Italy;*
[4] *Department of Neurology, Fundacion Jimenez Diaz, Madrid, Spain*

ABSTRACT

Twenty-four hour sleep patterns were measured in six healthy male volunteers during a 90-minute short sleep-wake (SW 30:60) cycle protocol for 48 hours. Sleep pressure estimates (amount of Slow Wave Sleep [SWS], SWA, and Rate of Synchronization [RoS: the rate of SWA build-up at the beginning of the NREM period]) were compared with the 24-hour patterns of body temperature (Tb_{24}) and sleep propensity. A moderate sleep debt was incurred over the 48 hour study as indicated by decreased levels of 24 hour sleep. On day 1, ultradian patterns of REM and SWS sleep were prominent; on day 2, more prominent were circadian patterns of REM sleep, SWS, Sleep Latency, TST and Tb_{24}. Also on Day 2, biphasic patterns of SWA and RoS were expressed, with peaks occurring during the falling and rising limbs of Tb_{24}. The biphasic peaks in SWA and RoS may be associated with phase-specific interactions of the circadian pacemaker with the sleep homeostat during conditions of moderate sleep pressure. Further research is needed to replicate the finding and to identify biological factors that may underlie the twelve hour pattern in SWA.

Key words

SWA • SWS • Rate of Synchronisation • Circadian rhythm • 30:60 sleep-wake schedule

Introduction

The human sleep-wake cycle is controlled by two central timekeeping mechanisms: a central circadian clock and a sleep homeostat. The primary circadian clock within the suprachiasmatic nucleus (SCN) generates a circadian signal that controls the circadian timing of behavioral and physiological rhythms, and also couples these processes to the outside world. The sleep homeostat maintains a day-to-day constancy in the level of sleep by determining current sleep needs (pressure) based on the history of prior wakefulness. One of the long established models of sleep regulation, the two-process model (Borbely, 1982; Daan et al., 1984; Achermann et al., 1993) proposes that slow wave activity (SWA) reflects the discharge of sleep pressure (hereafter referred to as 'S') that rises in proportion to prior wakefulness. During habitual sleep (i.e. 7-8 hours of sleep), sleep pressure (SWA, SWS) shows peak values shortly after sleep onset and then declines during successive nREM periods, reaching a lower asymptote after 4-5 sleep cycles (Feinberg, 1974; Dijk et al., 1990). Consistent with the function of the sleep homeostat, sleep deprivation (Dijk et al., 1993) and napping (Werth et al., 1996) increase and decrease, respectively, levels of S as reflected by the level SWA, and by the rate of appearance of SWA (RoS) at the beginning of sleep.

In some circumstances however, such as during extended sleep and during forced desynchrony studies, levels of SWA *rise* when sleep pressure

Corresponding Author: Wallace C. Duncan, Jr., Mood and Anxiety Disorders Program, NIMH, NIH, Bethesda, MD 20892, USA - Tel.: 301 496 9783 - Fax: 301 496 5439 - Email: wduncan@mail.nih.gov

† Deceased 18 July, 2003.

is presumed to be declining. Specifically, after about twelve hours, a second peak of SWS and SWA sometimes occurs during extended sleep (Webb, 1978; Gagnon et al., 1985; Broughton, 1989; Hayashi et al., 2002; Webb, 1986; Gagnon and De Koninck, 1984; De Koninck, 1996); enforced bed rest (Nobili et al., 1995); free running (Weitzman et al., 1980) and constant darkness (Hayashi et al., 2002). The mechanisms that control the late rise in SWA during these studies of extended sleep are unclear, but may be associated with the circadian clock, with sleep homeostasis, or with secondary circadian oscillators. Circadian modulation of SWA is evidenced in forced desynchrony studies where SWA exhibits a low amplitude circadian modulation that is controlled by the circadian clock (Dijk and Czeisler, 1995). This crest of SWA occurs on the rising limb of the circadian body temperature rhythm; the crest roughly corresponds to the midday rise of SWA and SWS that occurs during naps (Feinberg and March, 1995; Cajochen et al., 2004). The fact that SWA rises after an extended sleep episode suggests that non-homeostatic factors (i.e., other than prior wakefulness *per se*) might cause the paradoxical rise in SWA, or that an interaction between the circadian clock and the sleep homeostat might contribute to these increases in SWA (Dijk and Czeisler, 1995). In short photoperiod experiments (Wehr, 1992) and during sleep nap experiments in temporal isolation (Campbell and Zulley, 1985) human sleep is sometimes biphasic within a twenty-four hour period, i.e. with two symmetrical episodes of sleep separated by a period of increased wakefulness. Such studies suggest a more complex organization of the human sleep wake system, such that multiple oscillators, either within or outside the SCN, may affect the organization of the human sleep wake cycle (Wever, 1979; Masubuchi, et al., 2000; Inagaki et al., 2007).

The effects of the circadian pacemaker on sleep have previously been investigated using short sleep-wake cycle and/or forced desynchrony protocols in order to reduce or separate circadian from sleep homeostat effects. The short sleep-wake (S-W) cycle protocol (Kelley et al.,1973; Carskadon and Dement, 1975) decreases the influence of the sleep homeostat and enhances the influence of the circadian clock (Carskadon and Dement, 1980; Dantz et al., 1994; Tan et al., 2003). In this study a 90-minute S-W cycle paradigm was used to measure sleep stages, and two indices of sleep pressure, SWA and RoS (the rate of appearance of SWA within an episode of NREM sleep). Two questions were asked. First, are biphasic sleep patterns in SWS, SWA and RoS apparent in the short S-W cycle paradigm consistent with the presence of biphasic sleep patterns in habitual and other conditions? Second, are circadian rhythms (such as REM propensity and/or body temperature) associated with the temporal course of sleep pressure indices?

Methods

Subjects and protocol

Six normal male subjects (age: 20-29 years) participated in this exploratory study. Subjects had no personal or family history (first-degree relatives) of Axis I psychiatric illnesses, had normal physical examinations before the study and were not affected by any significant illness. Subjects were screened using a questionnaire that assessed life and sleep habits. Subjects gave written informed consent before participating in the study which was approved by the National Institute of Mental Health (Bethesda, MD) institutional review board. Individuals with sleep impairments or substance abuse were excluded from the study. Subjects were asked to complete a sleep log that assessed sleep onset and wake up in the weeks before the experimental session. Subjects maintained their habitual activity and sleep schedules and limited their daily caffeine consumption to no more than 3 caffeinated beverages per day during the two weeks preceding the study. No alcohol was permitted for the 2 weeks prior to the study. Subjects were asked to sleep according to a 30:60 sleep wake cycle for 48 hours starting at 23:00. The first experimental condition was preceded by a habitual sixteen hour day. Each 90-minute sleep-wake period consisted of a 30-min sleep opportunity (nap), followed by 60-min of reinforced wakefulness. During the wakefulness period, individuals were in constant dim light (< 1 lux) and were kept engaged by the nurse staff to avoid uncontrolled sleep episodes. Every 3 hours subjects were provided isocaloric meals. Alcohol and caffeine were not permitted during the study. Body temperature was recorded every 6 minutes using

an indwelling rectal probe connected to a portable electronic instrument that stored the measurements in electronic memory (Vitalog). The data were then transferred via an interface connection to a computer for analysis.

Polygraph recordings

Sleep was monitored with polygraphic recordings with a Grass 78 D polygraph. The half amplitude frequency of the high pass filter was set to 0.3 Hz for the EEG and EOG, and to 3 Hz for the EMG; the low pass filters was set to 35 Hz. A 4 Hz 25 microvolt calibration was used to verify signal amplitude. Sleep stages were visually scored according to Rechtschaffen and Kales (1968) criteria. For each sleep opportunity, sleep latencies to stage 1, stage 2, SWS and REM were defined as the time between lights off and the occurrence of the first 30-s epoch of each stage. EEG (C3-A2) signals were digitized on line (12 bit resolution, 102.4 Hz sampling rate) and stored on optical disk. Off line spectral analysis was performed on the EEG signals using a Fast Fourier Transform (FFT) routine (EEGSYS, Friends Medical Research Centre, Baltimore, MD; Schwartz et al., 2000). Power spectra were calculated for 5-s epochs using a 10% cosine tapered Hanning window and 0.2 Hz bands. Consecutive spectra were then averaged to yield 30-s spectra. Thirty-second power spectra contaminated by movement artefact were excluded from the SWA analysis. Epochs of stage 2, 3 and 4 were used to compute power spectra.

For RoS analysis, EEG power in the 0.4 to 4.4 Hz band for each 30-s epoch was averaged every minute and smoothed using two successive averages over a 5 point window (Fagioli et al., 1995, 2001). The time course of the smoothed spectral values was plotted against sleep stages. RoS (calculated for each NREM episode) was defined as the ratio of a) the difference between the *initial* delta power during the first minute of stage 2 and the *maximum* delta power measured later during the NREM episode and b) the latency (in minutes) from the beginning of the episode to the onset of the maximum delta power. RoS was computed only for sleep episodes in which delta power increased between the first minute of stage 2 and later in the NREM episode. 138 of the 192 thirty-minute sleep opportunities (6 subjects x 2 days x 16 sleep opportunities/day) contained sleep periods (as defined by occurrence of stage 2 lasting at least 3 minutes). The incidence of sleep periods in the six subjects ranged between 62.5-87.5%. Because multiple sleep periods sometimes occurred during the same opportunity, there were a total of 153 sleep periods. Only the sleep periods that displayed an increase of SWA after the beginning of the stage 2 (n = 142) were submitted to EEG RoS analysis. When more than one RoS event was observed in a sleep episode, the event with the higher RoS value was used. Thus, 138 sleep periods were submitted to the EEG analysis of RoS.

Statistics

ANOVA with two factors (*Day* [day 1 and 2] and *Time of Day* [16 scheduled sleep episodes]) was used to examine daily patterns of NREM sleep, SWS, REM sleep duration, stage 2 latency, SWA, RoS and temperature. For sleep episodes with no stage 2 sleep or REM sleep, sleep latency was defined as 30 minutes. Pairs of sleep episodes (1^{st} + 2^{nd}, 3^{rd} + 4^{th}, etc.) were combined by summing in order to adjust for empty cells in which REM sleep or SWS were absent; all subjects contributed to this analysis. Degrees of freedom were consequently halved. Data concerning EEG measures (SWA and RoS) were transformed (log (x + 1) using the recommended procedure when the data distribution are skewed and the measures are small (Myers, 1979, pp. 73). In this way a greater homogeneity of variance was achieved and the test power enhanced. Post hoc exploratory identification of peaks and troughs was conducted by comparing the sleep episode mean with the 48-hour mean of the same subject (t-test for paired sample). Post hoc exploratory analysis of sleep measures of day 1 in comparison with those of day 2 was conducted using a paired Student's t-test.

Estimation of period and amplitude

The period and amplitude of circadian rhythms was measured on day 2. Day 1 data was excluded since it likely retained the evoked effects of prior 24-hour light-dark cycles and sleep scheduling on sleep and temperature data. A relative score was derived by dividing each dependent variable by the 24-hour mean, and was then normalized using a log transformation. GraphPad PRISM software package version 2.0 (GraphPad Software, San Diego, CA, USA) was used for curve fitting and statistical analysis. Period, amplitude and phase estimates of sleep measures

during the 30':60' protocol, were derived using a minimal least squares algorithm to fit the day 2 twenty-four hour time series to a sinusoidal curve of the form $\{y(t) = M + A\sin(2\pi f t + P)\}$ where M = *mesor*, A = *amplitude*, f = *frequency*, and P = *phase*. The amplitudes of the fitted curves were evaluated using an F test; significance between fitted curves was calculated using an F-test criterion (GraphPad PRISM) that compared the sums of squares (SSs) of a non 24-hour complex model (unconstrained parameter values), with the SSs of a 24-hour model (constrained model; period = 24 h).

Results

The major differences between day 1 and 2 were a) increased stage 2 sleep latency ($P < 0.03$) and increased REM sleep latency ($P < 0.002$) on day 1 compared with day 2 (paired t test; Table I). There were trends of increased stage 1 sleep ($p = 0.077$) and decreased REM sleep duration ($p = 0.056$) on day 1 *versus* day 2 (Table I).

Significant *Time of Day* variation was found for NREM sleep (stages 2, 3, 4), REM sleep, stage 2 latency, SWS, SWA and body temperature (Tb) (two-way ANOVA; Table II, $p < 0.05$; Figg. 1 and 2). The 24 hour patterns of these variables were not different between days 1 and 2 (*Day* and *Time of Day*, $p > 0.1$). Several other sleep measures (TST, REM and RoS), exhibited different 24 hour patterns between days 1 and 2 (*Day* x *Time of Day* interaction, $p < 0.05$, Table II). TST peaked between 0630 and 1100, and reached a minimum level at 2300 on both days 1 and 2 (Fig. 1). REM sleep propensity peaked at 0630 on day 2.

Over the course of 48 hours, a reciprocal relationship existed between the level of Tb, and the 24 hour

Table I. - Day 1 *vs.* Day 2: comparison of sleep stages* and latencies*.

	Day 1		Day 2		t	p
	Mean	S.E.M.	Mean	S.E.M.		
Total Sleep Time (NREM + REM)	342	16	342	15	0.216	n.s.
SWS (stages 3 + 4)	26	5	30	7	0.232	n.s.
Stage 1	97	20	62	11	2.220	*0.077*
NREM sleep (stages 1 + 2 + 3 + 4)	296	9	268	15	0.700	n.s.
REM	46	14	74	10	2.479	*0.056*
LATENCY to stage 1	5	1	6	2	0.798	n.s.
LATENCY to stage 2	9	1	6	1	**3.009**	**0.030**
LATENCY to SWS	19	3	18	2	1.141	n.s.
LATENCY to REM	13	2	6	1	**5.841**	**0.002**

* Minutes
(two-tailed Student' t-test for paired samples: df. = 5)

Table II. - Time of day variation of sleep and body temperature.

	Day (1 *vs.* 2)		Time of day			Day x Time of day		
	$F_{1,5}$	p	$F_{15,75}$	$F_{7,35}$	p	$F_{15,75}$	$F_{7,35}$	p
Temperature	0.599	0.474, n.s.	7.485		**0.000**	1.232		0.268, n.s.
Total sleep time	0.001	0.979, n.s.	5.547		**0.000**	2.545		**0.004**
NREM (Stages 2 + SWS)	0.472	0.522, n.s.	2.728		**0.002**	0.747		0.729, n.s.
REM	6.143	0.056, n.s.		6.546	**0.000**		4.546	**0.001**
Stage 2 latency (corrected)	0.810	0.409, n.s.	4.394		**0.000**	1.393		0,173, n.s.
SWS	0.588	0.478, n.s.		2.321	**0.047**		1.593	0.170, n.s.
SWA (log)	0.725	0.427, n.s.	2.760		**0.002**	1.180		0.306, n.s.
RoS (log)	0.630	0.463, n.s.	1.941		**0.032**	1.979		**0.028**

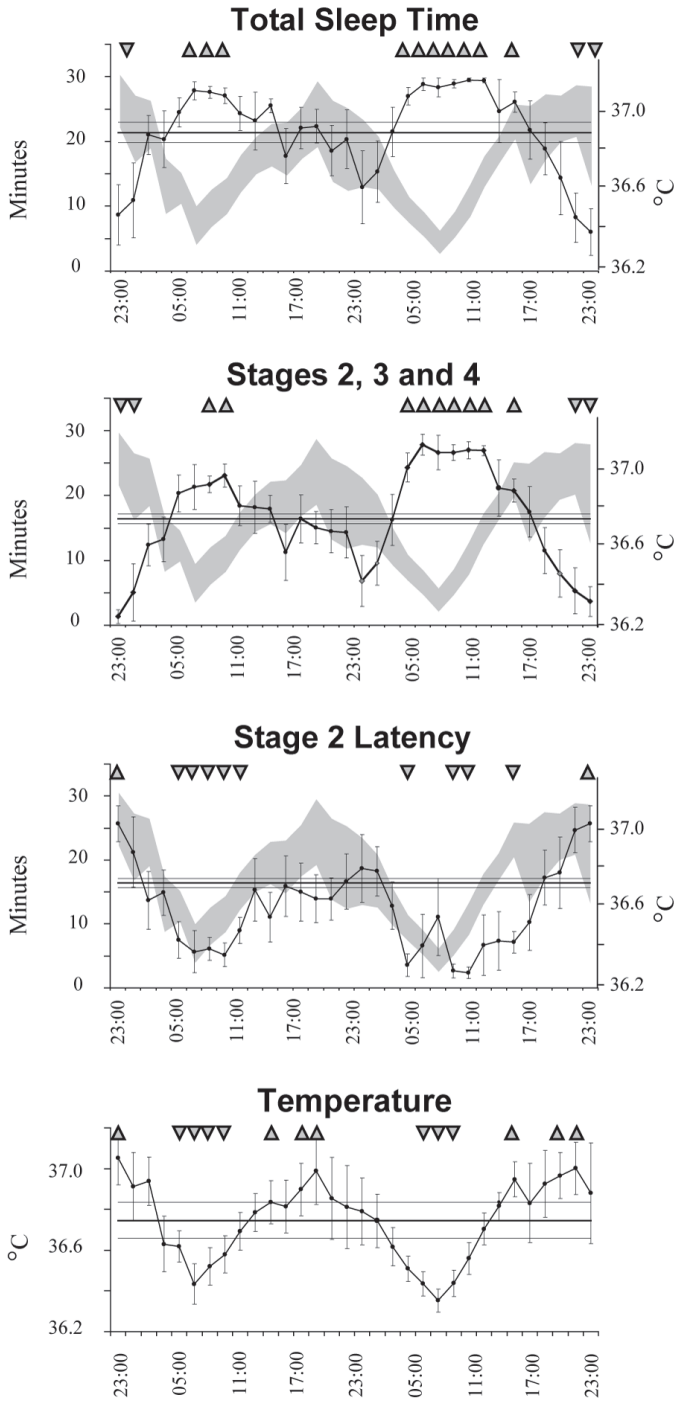

Fig. 1. - Group averages (mean ± sem; n = 6) of total sleep, NREM sleep, sleep latency and body temperature during each thirty minute sleep opportunity of the two day 30:60 sleep wake schedule are shown. Clock time is indicated on the x-axis; Tb level(degrees centigrade) is indicated on the second Y-axis. On each panel, the thick horizontal line indicates the group mean for the entire study period. The two thin lines above and below the group mean indicate the standard error of the mean. Individual values that deviate significantly from the two-day mean are indicated by the triangles above each figure. In each panel, the gray region depicts the mean (± sem) body temperature level also displayed in the lower panel.

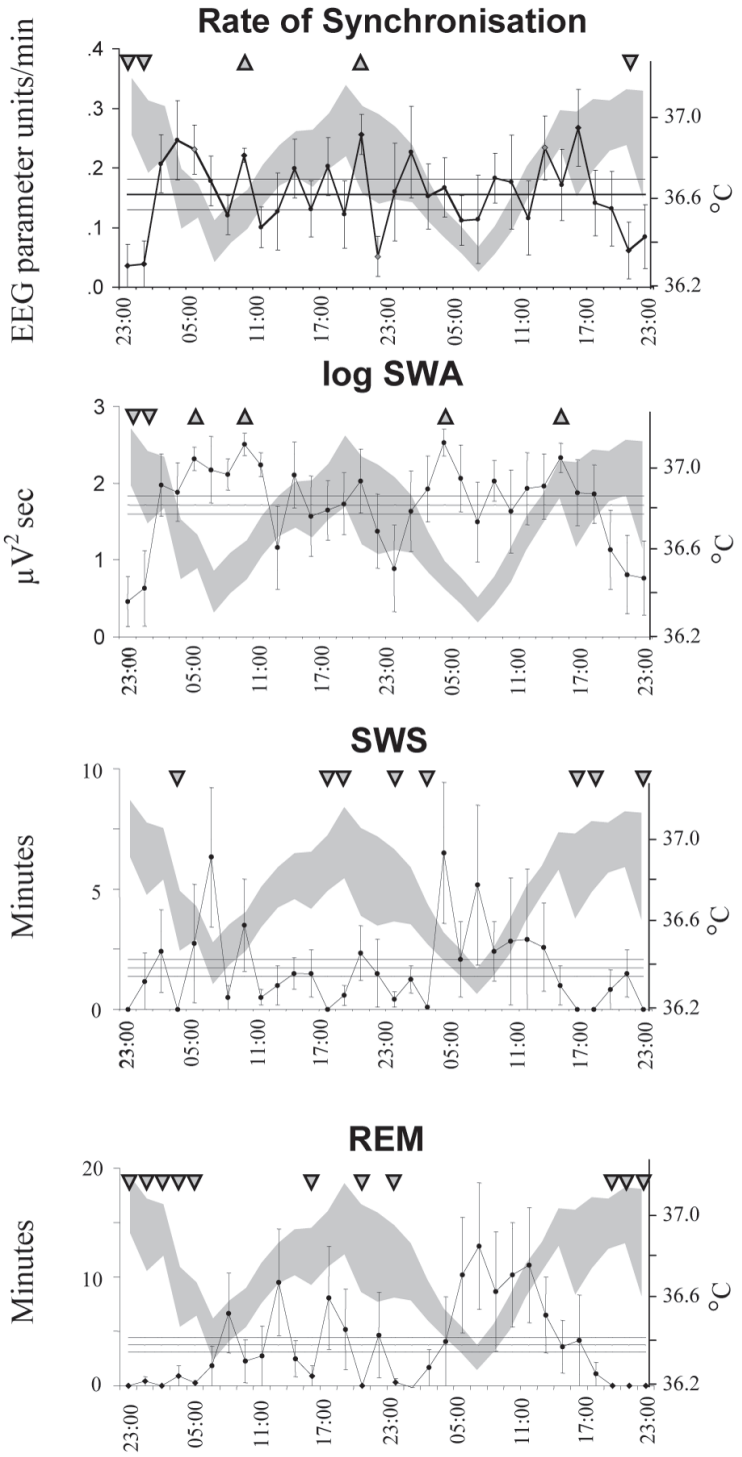

Fig. 2. - Group averages (mean ± sem; n = 6) of RoS (Rate of Synchronisation), log SWA (slow wave activity), SWS (slow wave sleep) and REM sleep, during each thirty minute sleep opportunity of the two day 30:60 sleep wake schedule are shown (see Fig. 1 for further details).

pattern of many sleep measures (TST, NREM duration, SWA, SWS and REM sleep duration; Figg. 1 and 2). Close inspection of the data indicated a complex ultradian relationship between Tb, SWS and REM on day 1, and between Tb, SWA and RoS on day 2. During naps on day 1, REM and SWS cycled in an alternating pattern. On day 2, SWA and RoS exhibited a biphasic pattern. These patterns are discussed in greater detail in the following sections.

Cycles of SWS and REM sleep (day 1)

On day 1, elevated levels of REM sleep were observed on every third, 30 minute sleep opportunity. These naps alternated with 2 naps that contained elevated SWS and low REM content (Fig. 3). Analysis of sleep stage frequency indicated that SWS and REM sleep segregated during the different sleep opportunities. 60 of the 192 sleep opportunities showed SWS with no REM sleep, 44 showed REM sleep with no SWS. In only 3 of 192 cases did both SWS and REM sleep occur during the same sleep opportunity ($Chi2 = 19.733$, $p < 0.001$). The alternating pattern of SWS and REM was significant on day 1 (Fig. 3) when two consecutive sleep opportunities with low REM was followed by a third opportunity with elevated REM (repeated-measures ANOVA; $F = 4.09$, $df = 2, 17$; $p = 0.05$; Fig. 3). Post-hoc analysis indicated lower SWS levels during sleep opportunities when REM was elevated ($t = 2.535$, $p < 0.05$); this pattern was not evident for SWA.

24- and 12-hour patterns of sleep (day 2)

Circadian patterns of SWS and REM sleep were more pronounced on day 2 than on day 1 (Fig. 2). On day 2, REM and SWS levels increased between 0330 and 0930, when Tb was below its 24-hour mean. REM and SWS levels decreased after 1100 when Tb was above its 24-hour mean (Fig. 2). Sleep Latency peaked between 2300 and 0030 when Tb was declining. REM, SWS, Sleep Latency and Tb patterns were fit by unconstrained cosine curves with periods of 20.8, 22.9, 25.7, and 23.2 hours, respectively. An F test that compared the fit of these

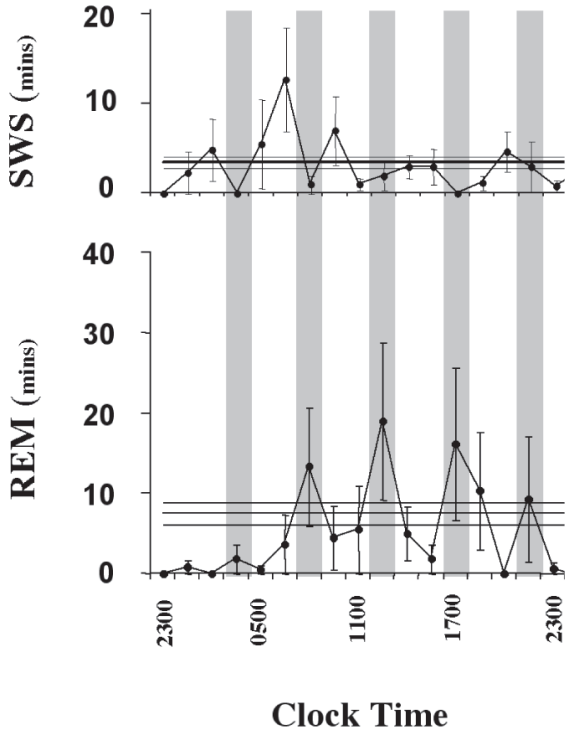

Fig. 3. - Minutes of SWS and REM sleep are shown for each scheduled sleep opportunity on day 1. Note the cycling pattern between SWS and REM in which REM peaks every third sleep opportunity (vertical grey rectangles). SWS levels are lower when REM levels are high ($p < 0.05$).

periods with a 24 hour period indicated no significant difference between the unconstrained and 24 hour curve estimates (REM and SWS, Fig. 4).

In contrast, SWA and RoS appeared in a biphasic pattern on day 2. The first SWA and RoS peaks occurred from 0300-0500; the second peaks occurred from 1500-1700 (Fig. 2). SWA and RoS data were fit by unconstrained cosine curves with periods of 11.0 and 11.9 hours respectively (Fig. 4). These period estimates differed significantly from 24 hours (SWA, $F = 4.92$, $p = 0.029$; RoS, $F = 4.81$, $p = 0.028$). The timing of the first (night time) peaks in SWA and RoS was after a six-hour interval of lowTST and high waking (2000-0200 h) and prior to the Tb trough. The second (mid-day) peaks occurred after an interval of high REM and TST and low waking (0500-1100), and prior to the Tb peak (6:30-8 PM; Fig. 2).

Discussion

Well described circadian rhythms of sleep latency, REM sleep, and TST were observed during this 90 minute short S-W cycle protocol. Previously unreported was the day 2 appearance of a biphasic pattern in SWA and RoS that complement reports of biphasic SWS and SWA patterns during numerous and varied extended sleep and nap protocols (Webb, 1978; Weitzman et al., 1980; Gagnon and De Koninck, 1984; Webb, 1986; Nobili et al., 1995; De Konick, 1996; Christ et al., 1996; Hayashi et al., 2002; Cajochen et al., 2004; Munch et al., 2005). In contrast, a biphasic pattern of SWA was not observed in forced desynchrony (FD) (Dijk and Czeisler, 1995), in a 90 minute (Niggemyer et al., 2004) or in a 225 minute short S-W cycle protocol (Knoblauch et al., 2003). The different designs and details in the execution of FD protocols, as well as between apparently similar short S-W paradigms may account for some of these differences. For example, the onset of the current experiment at 2300 after 16 hours of wakefulness would produce a greater sleep dept during the next day than short-day experiments that began at 0800 (Niggemyer et al., 2004; Knoblauch et al., 2003), as would a FD protocol in which the sleep period was less than one-third of the programmed sleep-wake period. The initial levels of nREM and REM sleep pressure, as well as their interaction with circadian propensities to alter their levels, may distinguish the findings of this study from others.

This short sleep wake protocol replicated many of the sleep patterns observed in earlier short S-W cycle studies (Carskadon and Dement, 1975; Kudo et al., 1999; Tan et al., 2003). The peak of the circadian rhythm of sleep duration coincided with the trough of the daily body temperature rhythm; the trough of sleep coincided with the evening wake maintenance zone (Fig. 1a), and with the long sleep latencies during this zone (~2300). Sleep latency exhibited a steep decline after the wake maintenance zone, followed by a rise after the trough in Tb (Fig. 1c). An ultradian rhythm of REM and SWS was prominent with REM and SWS exhibiting a nearly mutually exclusive and periodic pattern (Fig. 3), as described earlier (Carskadon and Dement, 1975; Dantz et al., 1994). Of particular importance, total sleep averaged 342 minutes on days 1 and 2, resulting in a progressive sleep debt present in many short sleep-wake protocols (Carskadon and Dement, 1975; Niggemyer et al., 2004). The shortened 30 minute sleep episodes may underlie the reduced production of SWA compared with levels observed during habitual sleep (Achermann et al., 1993). Consequently, in addition to the role of the circadian pacemaker, the influence of the sleep homeostat and the moderate increase in (nREM and REM) sleep pressure must be considered when interpreting these data.

The twenty-four hour REM sleep deficit (Table I) would be expected to increase the homeostatic pressure for REM sleep on day 1 and 2 (Beersma et al., 1990; Brunner et al., 1990). The rebound on day 2 of REM sleep may be related to the compensatory interaction of different levels of nREM and REM sleep pressure during the two day protocol (Beersma et al., 1990). On night 1, the short 30' sleep episodes led to deficits of both nREM and REM sleep; the consequent late night increase in nREM sleep pressure may have inhibited and blunted the expression of REM relative to the Tb trough on day 1, consistent with inhibitory effect of the sleep homeostat on REM sleep during initial sleep (Dijk and Czeisler, 1995). In contrast to day 1, on day 2, the circadian rhythm of (REM) sleep propensity, which peaked during the temperature trough (Fig. 2d), is more prominent. It would appear that on day two, a sleep

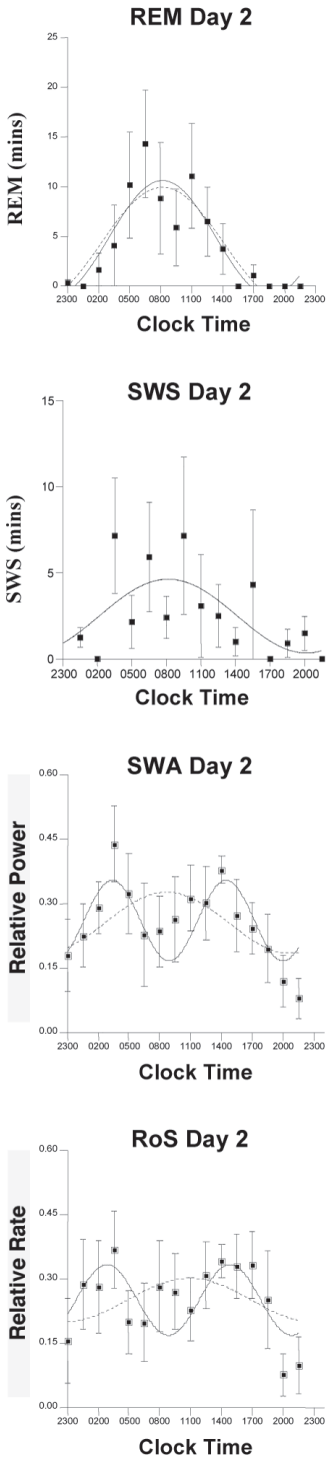

Fig. 4. - The best-fit curves for REM sleep, SWS, SWA, RoS, during day 2 of 30:60 sleep wake schedule are shown (solid lines). Also shown are the curves constrained to 24 hours (dashed lines; the SWS curve is not visible). Note the circadian pattern of REM and SWS in contrast to the twelve-hour pattern for both SWA and RoS. Clock time is indicated on the x-axis. The group average for each time point is indicated by the filled square. The vertical bars represent the SEM at each time point. The data were fit to sine function of the form $\{y(t) = M + A\sin(2\pi f t + P)\}$ where *M = mesor*, *A = amplitude*, *f = frequency*, and *P = phase*. REM and SWS have circadian periods of 20.8 and 22.9 hours, respectively. SWA and RoS have periods (significantly different from 24 hours) of 11.04 and 11.95 hours, respectively. The second peaks for both SWA and RoS follow the circadian peaks for REM and SWS.

homeostat dependent disinhibition of REM sleep might contribute to the peak of REM propensity since SWA was elevated prior to the increase in the peak of REM propensity. Since the peak of REM sleep propensity is driven by the circadian pacemaker (Dijk and Czeisler, 1995), the day 2 biphasic patterns of SWA/RoS may be related to the interaction of the circadian rhythm of REM sleep propensity with the homeostatic regulation of SWA/RoS.

The fact that both SWA and RoS exhibit a similar biphasic pattern is consistent with their common regulation by 'S'. In the two process model of sleep regulation, SWA and RoS are predicted to change together since the level of SWA that occurs within each nREM period, as well as the rate of build-up of SWA within a nREM period (RoS in the current study), are both determined by the level of S, which increases in proportion to the amount of prior waking (Achermann et al., 1993). In the revised version of the sleep model, the level of S is also dependent on EEG sleep stages (such as REM and low SWA and nREM sleep states). After prolonged daytime wakefulness, the beginning of habitual sleep is characterized by elevated SWA and RoS which then exhibit a progressive homeostatic decline together as sleep progresses (Achermann et al., 1993; Fagioli, et al., 2001). However, the appearance of a biphasic pattern also suggests a more complex regulation of SWA than that indicated by a simple homeostatic decline. One might consider an effect of a) non-waking sleep stages on SWA, and/or b) an effect of other circadian or oscillatory networks as contributing factors to the biphasic pattern.

A technical point should be raised prior to further discussion about timing of some EEG events because the peaks and troughs of SWS, SWA and RoS on day 2 (Fig. 2) do not consistently overlap in this study as one might initially expect. We attribute such timing discrepancies to the different analytical techniques for measuring these variables. Specifically, visual *versus* computerized estimates of SWS and SWA sometimes diverge (see Fig. 10, in Achermann et al., 1993; Beersma et al., 1990) thus affecting the level and timing of SWA/SWS peaks. In the current data, at 2 PM on Day 2, low and moderate amounts of SWA contribute to the SWA peak at 2 PM; these peaks contrast with the slow wave features that are apparently below threshold criteria for visually scored SWS. Also, with regard to the different timing of the SWA and Ros peaks, although RoS and SWA are both markers of "S", RoS includes a time dimension (i.e. the latency to the maximum delta wave amplitude) which affects the value of RoS. Therefore, low and moderate levels of SWA appearing shortly after the onset of nREM sleep might compute to large RoS values. Overall, the variability in the circadian timing of these variables illustrates the dynamic structure of SWA expression within a nREM period.

The timing of biphasic peaks of slow wave activity

The biphasic SWA and RoS peaks described here coincided with the falling and rising limbs of Tb_{24}. For discussion purposes, the two peaks will therefore be referred to as the "night time" and "daytime" peaks, because these Tb limbs are generally associated with the dark and light phases of the circadian cycle, respectively. As discussed below, the timing of the SWA peaks are similar with those reported in many prior studies.

The timing of the *daytime peak* of SWA corresponds with the crest and trough of the rhythms of NREM and REM sleep respectively (Dijk and Czeisler, 1995), as well as with the peak of daytime naps and the dip in afternoon performance. The daytime peak is thus consistent with varied experimental protocols such as a) the timing of the SWA crest in FD (Dijk and Czeisler, 1995), b) the rise in mid-day SWA 2-3 hours prior to the Tb crest during prolonged sleep (Table 2, Christ et al., 1996), c) the increased rate of delta (1-4 Hz) power production during naps of *individual* subjects between 1200-1800 h (Feinberg and March, 1995), and d) the afternoon peak in SWS measured during naps in a 225 minute short S-W cycle protocol (Knoblauch, 2003; Cajochen et al., 2004; Munch et al., 2005). The daytime peak in SWA follows the circadian peak of REM and sleep propensity by 8 hours (Fig. 4); the SWA peak could therefore be delayed by the interaction of a) the circadian peak in REM sleep propensity and b) increased REM sleep pressure that suppresses nREM intensity (Beersma et al., 1990). However, the timing of the daytime peak in SWA is notable because it coincides with reported circadian pacemaker modulation of the daytime peak of SWA and nREM in FD (Dijk and Czeisler, 1995), but in the absence of a REM sleep deficit. This timing sug-

gests that elevated REM pressure is less critical than is the circadian pacemaker phase (the late rising limb of Tb) for the rise of SWA.

The timing of the *night time peak* in SWA closely follows the peak of the circadian drive for wakefulness, and precedes the circadian peak of REM sleep by 6 hours. This night time peak in SWA corresponds with the opening of a circadian "gate to sleep" (Lavie, 1986), as well as with increased subjective sleepiness on the Karolinska Sleepiness Scale (Cajochen et al., 2004). The night time peak in SWA corresponds with night time peaks in delta activity during a 60 minute short S-W cycle protocol (Tan et al., 2003) as well as with a peak of SWS in a 225 minute short S-W cycle protocol (Cajochen et al., 2004). In contrast to the moderate "daytime" crest in SWA discussed above, a "night time" SWA peak is not observed in FD (Dijk and Czeisler, 1995); there are several possible explanations for these different findings. The circadian rhythms of sleep propensity, melatonin and REM sleep might contribute to the relative size of the night time and daytime peaks as follows. Just as the crest of daytime SWA (on the rising Tb limb) might be attributed to *decreased* sleep time (attributed to the circadian rhythm of sleep propensity) in the prior sleep episode (Dijk and Czeisler, 1995), a flat "night time" SWA profile (on the falling Tb limb) might conversely be attributed to *increased* sleep time during prior FD sleep episodes (if this were true, an FD protocol designed to increase the sleep deficit might induce a night time crest in SWA). To test the idea that the level of SWA might be related to prior waking and/or REM duration in our day 2 data, total SWA from 11 AM and 11 PM (the hours of elevated SWA), was correlated with a) total REM sleep, and b) REM sleep plus awake from 11 PM and 11 AM (the hours of elevated REM). Total SWA was negatively correlated with REM duration and REM duration plus awake ($r = -0.37$, $r = -0.59$ respectively). On day 2, SWA was not correlated with prior wakefulness during the preceding two-hour interval ($r = -0.198$). The lack of correlation may be related to the small sample size, or to more complex circadian processes that affect SWA. An effect of melatonin should also be considered, since melatonin diminishes night time SWA power density relative to daytime SWA (Dijk et al., 1997); thus daytime and night time SWA crests would be augmented and diminished respectively. Whether modulation of SWA could be attributed to REM sleep variation has been considered (see discussion in Dijk and Czeisler, 1995). As discussed below, in this short S-W cycle experiment, REM sleep may also have contributed to the biphasic SWA pattern.

Extended night experiments (Wehr, 1992) provide further clues regarding the complex relationships between prior waking, sleep debt and REM homeostasis on the one hand, and the biphasic pattern of SWA and RoS on the other hand. During the extended night (short photoperiod; L:D 10:14) sleep paradigm, human sleep often becomes biphasic with two distinct sleep bouts separated by an interval of wakefulness. The biphasic timing of the sleep and SWA/RoS profiles in the extended night and short sleep experiments are somewhat similar. During extended night, biphasic sleep bouts occurs on the falling and rising limbs of the Tb rhythm (Wehr, 1992), similar to the biphasic short S-W cycle SWA/RoS patterns. Also biphasic sleep patterns during extended night are sometimes characterized by elevated sleep pressure, as indicated by increased RoS (Fagioli et al., 2001). The fact that the biphasic bouts are separated by waking in the extended night experiment, whereas crests of SWA and RoS are separated by REM sleep in the short S-W cycle experiment, is consistent with the predicted effects of waking and REM sleep on the level of sleep pressure and SWA in the *revised* two process model (Achermann et al., 1993). The similar timing relative to the rising and falling limbs of Tb, of the biphasic sleep bouts and SWA/RoS peaks in the short sleep and extended night experiments, points to similar circadian biology that underlies these events.

Multiple oscillators, both within and outside of the suprachiasmatic nucleus, affect the organization of the mammalian circadian system (Wehr, 1992; Masubuchi et al., 2000; Wehr et al., 2001). In humans, when photoperiod duration is experimentally manipulated as in the extended night experiment described above, the rise and fall of numerous measures including melatonin secretion, Tb_{24} and REM sleep, each resynchronize to the new dusk and dawn transitions (Wehr et al., 2001). The dusk and dawn transitions are in accord with a circadian pacemaker two oscillator system (Jagota et al., 2000; Daan et al., 2001) in which a morning and evening oscillator separately synchronize to dusk and dawn transitions, in order to accommodate seasonal change in day length. The neurophysiological

basis for the two oscillator model has recently been revealed within the mammalian circadian pacemaker (Inagaki, 2007). One might conjecture that these two oscillators, previously linked to the rise and fall of Tb_{24} (Wehr et al., 2001), might contribute to the timing of the biphasic patterns of SWA and RoS.

Recent experiments have also shown that SWA is related not only to the quantity of prior waking history, but also to the quality of the behavioural history (Kattler et al., 1994; Vyazovskiy et al., 2000; Huber et al 2004, 2006). The contribution of *qualitative* aspects of waking history (as assessed by psychomotor vigilance testing for example) was not addressed during this exploratory work, but may have contributed to the biphasic SWA pattern. The relationship between these aspects of prior waking and the biphasic SWA pattern could be explored in future experiments.

In summary, this short S-W cycle protocol provides preliminary evidence of a biphasic pattern of SWA/RoS; the biphasic pattern is characterized by a "night time" and a "daytime" crest of SWA/RoS that coincides with the falling and rising limbs of Tb. The night time peak appears to be dependent upon increased sleep pressure incurred by the sleep homeostat due to abbreviated sleep cycles during the short S-W cycle protocol. The biphasic pattern of SWA is suggested to be linked to an interaction between the sleep homeostat and the circadian pacemaker, and would especially be evident as daytime napping during habitual sleep when a sleep debt is present. As such, the findings suggest temporal details of circadian pacemaker control of SWA that could modulate the functional and restorative aspects of human sleep.

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