SLEEP-WAKE, ENDOCRINE AND TEMPERATURE RHYTHMS IN MAN DURING TEMPORAL ISOLATION

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In 1729 de Mairan first reported to the French Royal Academy of Sciences in Paris, that a biological organism (a "sensitive plant") would continue to have a 24 hour rhythm of activity when light-dark entraining stimuli were absent. De Candolle, in 1832 first described a free-running rhythm in the same species of plant with a progressive phase advance of 1.5 to 2 hours per day. Subsequent studies have demonstrated that organisms not entrained by 24 hour "zeitgebers" (time cues), develop daily cycles with periods greater or less than 24 hours (Pittendrigh, 1961). Extensive research in animals utilizing a rest-activity measurement has demonstrated that these "free-running" period lengths are species-specific and genetically influenced. When an animal is maintained in constant conditions the new cycle length can be remarkably constant for months to several years (Daan & Pittendrigh, 1976; Pittendrigh & Daan, 1974; Pittendrigh & Daan, 1976a; Pittendrigh & Daan, 1976b). In 1962, Aschoff and Wever did their studies on three men and three women living for 8 to 19 days in a "deep cellar" in Munich, and first demonstrated that normal man would also maintain a "circadian" activity-rest cycle which would "free-run" with a non-24 hour period when isolated from all time cues. Many subsequent studies have repeatedly confirmed those observations in man (Mills,1966; Mills, 1974; Mills, Minors, & Waterhouse, 1974; Mills, Minors, & Waterhouse, 1976), and have extended the measurements to include body temperature, urinary electrolytes and certain hormonal metabolic products (Aschoff, 1967; Aschoff, 1969; Aschoff, 1970; Aschoff & Wever, 1976; Aschoff & Wever, 1976; Aschoff, Gerecke, & Wever, 1976). In almost all instances of several hundred such studies now performed (Chouvet, Mouret, Coindet, Siffre, & Jouvet, 1974; Jouvet, Mouret, Chouvet, & Siffre, 1974; Siffre, 1965; Siffre, Reinberg, Halberg, Chata, Perdriel, & Sling, 1966; Webb & Agnew, 1972; Webb & Agnew, 1974a; Webb & Agnew, 1974b) including cave and controlled laboratory environments, the period length of such activity-rest rhythms have been greater than 24 hours, typically occurring at approximately 25 hours. Important conclusions have been arrived at, such as the change of phase angle relationship between body temperature and rest time (Aschoff et al., 1976; Chouvet et al., 1974; Jouvet et al., 1974; Siffre, 1965; Siffre et al., 1966; Webb & Agnew, 1972; Webb & Agnew, 1974a; Webb & Agnew, 1974b; Wever, 1973), the ability of different variables to develop independent cycle lengths during free-running (Aschoff, 1973; Aschoff, Gerecke, & Wever, 1967), and the concept of multiple oscillators normally synchronized with each other but which can become desynchronized under free-running conditions (Wever, 1975; Wever, 1977). The importance of "social" entraining rather than light-dark cues for man has been emphasized (Aschoff, Gerecke, Kureck, Pohl, Reiger, Saint Paul, & Wever, 1971), and in some subjects the ability to develop and sustain very long rest-activity periods (between 30 and 50 hours in length) has been recognized (Mills et al., 1974; Aschoff, 1967; Chouvet et al., 1974; Jouvet et al., 1974; Findley, Mialer, & Brady, 1963).

It has been assumed until recently that the "rest" segment is a sleep period in these studies determined either by "lights out", absence of activity, or "bed-rest" time. Except for the cave studies of Jouvet et al. (1974), and the short "isoloation" studies of Webb et al. (1972; 1974a; 1974b), systematic studies of the temporal complexity of polygraphically defined sleep stages have not been reported. Analysis of the previously reported detailed sequential durations of the "rest" (selected lights out) time indicates that a significant day to day variability is present in almost all subjects, suggesting that interval sleep stage amounts and timing may be related to such variability. The previous assumption that "rest" is sleep cannot be made. These events alter biological rhythm cyclic properties and will influence other correlative measured periodic events such as body temperature and hormonal cycles. All previously reported studies have maintained subjects in time-free environments totally isolated from direct human contacts during the duration of their stay. We considered it important to study subjects in temporal isolation but with human social communication. This has the major advantage of allowing us to make certain biological measurements and psychological observations not possible with the previous constraints. Finally, all previous studies of hormonal cycles in temporal isolation studies have only used urinary measurements of derived metabolic products (Aschoff & Wever, 1962; Aschoff, 1967). In a series of recent studies (Weitzman, Schaumburg, & Fishbein, 1966; Weitzman, Fukushima, Nogeire, Roffwarg, Gallagher, & Hellman, 1971; Weitzman, Boyar, Kapen, & Hellman, 1975; Weitzman, 1976; Hellman, Nakada, Curti, Weitzman, Kream, Roffwarg, Ellman, Fukushima, & Gallagher, 1970), we had developed methods of obtaining frequent plasma samples, and demonstrated that important relationships exist between hormonal blood concentrations, sleep, and sleep stages.

We have carried out detailed and prolonged measurements of sleep-waking function in human subjects for time periods ranging from 25 days to 6 months. We measured polygraphic sleep-stage characteristics, minute by minute body temperatures and frequent (approximately 20 minutes) blood sampling for cortisol and growth hormone in normal adult men living in an environment free of all time cues, under entrained, free-running and re-entrained conditions. The results described are part of a comprehensive multi-variable study of the chronophysiology of man living in a time free environment with a non-scheduled daily pattern of living.

Methods

A special environment was established where the individual subjects lived for many weeks. A three room apartment (study, bedroom, and bathroom) was arranged without windows, the walls sound attenuated and a double door entrance to the temporal isolation facility (TIF). A closed circuit TV system and voice intercom monitored the subject's activities.

Ten male subjects were individually studied. The first group (3 subjects, FR 1, 2, & 3) was studied for 15 calendar days and the second group (6 subjects, FR 4, 5, 6, 7, 9, & 10) for 25 calendar days and a single subject (PR 1) for an extended stay of 105 calendar days. No subject had significant psychopathology, medical illness, nor were any on drugs. Each subject kept a written daily diary of sleep times for at least 2 weeks and maintained a regular scheduled sleep-wake schedule in accord with their usual habits. After

entry in the TIF, an entrained condition of 3 or 4 scheduled 24 hour sleep-wake periods preceded the non-scheduled "free-running" portion of the study. The entrained clock times was determined by the subject's recorded habitual lights off-lights on time at home. The subject was told that his sleep time would be scheduled for certain portions of the study but was not advised of the clock times nor the duration. Following the entrained portion, each subject was told that he could choose to go to sleep and awaken at any time he wishes. He was not allowed to "nap". A decision to go to sleep, therefore, represented the sleep period for that biologic "day". Food was available to the subject on demand as breakfast, lunch, dinner, and a "snack". The subject could request any meal type at any time. A set of buttons were available which when pushed were coded on a paper punch tape and indicated the behavior the subject was about to initiate and the elapsed time (to the nearest minute) from the beginning of the study. These behaviors included meal and type, sleep time, awake time, urinating, taking showers, defecating, taking blood samples, and exercising. The paper punch tape structured the entire time series of each study.

The subject was totally isolated from contact with all non-laboratory persons but communicated by intercom and direct discussion with selected laboratory staff. The supervising staff members were scheduled on a random basis as to time of day and duration of work-shift to prevent the subject from obtaining time cues.

The following measurements were made for each subject:

(1) Polygraph-Sleep Recording--The interval between the subject's decision to sleep and light-out with full electrode application for polygraphic recording was less than 15 minutes. All polygraphic records were scored by standard methods (Rechtschaffen & Kales, 1968).

(2) Rectal Temperature--A rectal thermistor probe was maintained by each subject throughout the entire study except for brief daily periods of defecation. The temperature was automatically recorded every minute on the punch paper tape and a print-out.

(3) Plasma Cortisol and Growth Hormones--A catheter with 3 holes at the tip instead of the usual one, was inserted into an arm vein of nine subjects at the start. At approximately 20 minutes, sampling of blood was obtained. This venous catheter was changed at 2-5 day intervals using alternating arm veins without interrupting the sampling. Subject PR 1 did not have blood samples obtained. Plasma cortisol assays were performed using the competitive protein binding technique (Murphy, Engelberg, & Pattee, 1963). The samples were assayed in duplicate using 25ul aliquot for each assay. HGH was assayed in duplicate from each plasma sample by radioimmunoassay using 20ul of plasma for each assay.

(4) Polygraphic Data Scoring--All scored data were transferred to a computer compatible format and analyzed for total sleep, lights out, and all sleep stages for each lights out-sleep period. The pattern of sleep stage sequences was visualized by a special display program. A quantitative determination was made for a set time period of the percent of each sleep stage and waking. The result of that analysis was also displayed utilizing a computer plotting technique.

(5) Special Mathematical Techniques and Computer Algorithms--In addition to the usual statistical method of analysis and computer plotting and display routines, several mathematical techniques were created to assist in the analysis of the data. These include a) estimate of period length using a minimum variance fit, b) wave form eduction and c) averaged time locked response.

Results

Activity-rest Cycle and Sleep Stages

Each of the 10 subjects developed a free-running sleep-wake cycle following the entrained baseline condition. In each case the mean period length was longer than 24 hours (Table 1). The subject population was divided into two types (excluding the tenth subject (PR 1)). In Type A, (6 subjects - FR 1, 2, 5, 6, 7, 9) the period lengths during FR averaged between 24.4 and 26.2 hours, whereas Type B (3 subjects, FR 3, 4, 10) had consistently long periods greater than 37 hours. The lights-out period for the Type B subjects ranged from 8 to 20 hours, with an average of 14 hours. Linear regression analysis through mid-sleep times demonstrated a very stable period length, (r^2.99 for each).

Table 1

Total Sleep Time (Mins) REM % and 3 + 4% of Total Sleep Time of Subjects During the Three Experimental Conditions

Entrained	FR 1	FR 2	FR 3	FR 4	FR 5	FR 6	FR 7	FR 9	FR 10
TST	435	398	466	448	443	446	390	423	401
REM %	28%	18%	30%	13%	26%	24%	21%	19%	15%
3 + 4%	18%	18%	25%	27%	24%	26%	39%	28%	36%
Free-running									
TST	483	376	830	770	445	448	373	364	584
REM %	25%	22%	28%	15%	26%	25%	25%	17%	15%
3 + 4%	25%	24%	19%	17%	26%	34%	31%	37%	33%
Re-entrained									
TST				454	412	397	366	398	
REM %				15%	26%	25%	24%	14%	
3 + 4%				24%	25%	41%	31%	35%	

Short sleep periods recurred at a regular phase of the circadian cycle with a period slightly longer than 24 hours. The long sleep periods began at a phase angle approximately 180 degrees shifted from that of the short sleep periods.

Variation in sleep lengths were related to the phase of the ongoing circadian oscillation at which the sleep period occurs. When prior wakefulness lasted more than 1440 minutes, there was a clear increase of sleep length with episodes lasting 600 to 1200 minutes.

Subject PR 1, the 10th subject, lived under "free-running" condition for 80 calendar days and demonstrated several important features. He maintained a regular free-running period length of approximately 25 hours for the first 30 activity-rest cycles. He then developed an activity-rest cycle pattern consisting of alternating long cycles (36 hours) with a series of shorter cycles (approximately 25 hours). This alternating pattern persisted until it was interrupted by a special light-dark entrainment protocol on calendar day 84. The sleep time continued on an approximately 25 hour period length in spite of the interruption by very long non-circadian periods. These approximately 25 hour self-selected sleep-wake times were therefore entrained to an internal periodic process, which can be considered an "internal zeitgeber". The long sleep periods (600 minutes) occurred at a phase angle approximately 180 degrees shifted from the short sleep periods but in parallel with the same period-length mid-sleep regression line. Analysis of the relationship between length of sleep period and length of prior wakefulness demonstrated that for only 7 out of 16 waking periods lasting greater than 20 hours, did the subsequent sleep period exceed 12 hours in length. However, as was the case for the other subjects, no long sleep period was preceded by a wake period less than 20 hours in length.

There was a rapid phase delay of lights-out and sleep onset of at least 6 hours within 48 hours of the onset of the free-running condition for 8 of the 9 subjects. The ninth subject (FR 7) delayed his sleep onset by 5 hours on the third biologic day. In addition, all 6 subjects in Group A had a characteristic "scalloped" appearance of the timing of lights-out with a variable cycle of 3-4 days. This could not be explained as a "transient" process related to the onset of FR since it clearly continued throughout the FR condition in four subjects (FR 2, 5, 7, 9).

The lights-out period in general corresponded with the sleep period for each subject and for each night. However, it was found that at times, there was a short delay from lights-out to sleep onset. The two older subjects (ages 50, 51) (FR 9 and FR 10) consistently interrupted their sleep periods by awakening for short periods during the subjective night as well as remain awake in the dark for periods up to one hour after awakening and prior to signaling "lights on". These waking interruptions were also present during the entrained segment as well. These findings emphasize the importance of defining sleep stages polygraphically when measurements of biological ryhthm variables are made.

There was considerable variability in the mean total sleep time (TST) during FR with two subjects averaging 13.8 and 12.8 hours (FR 3,FR 4)(Table 1). In spite of this variability in total sleep time per sleep period during FR, the ratio of sleep time to period length only varied between .24 and.35 across subjects with an average of .29. This compared with .30 during the entrained condition. When the entrained ratio was compared to the FR ratio for each subject, it was noted that two subjects with high entrained ratios (long sleepers)(.31 and .32) increased the value to .35 and .34 respectively, during

free-running, whereas four subjects with the lowest entrained ratios (.27, .28, .29, and .29) (short sleepers) all decreased the ratio to .25, .25, .24, and .25, respectively, during FR. The 3 other subjects with intermediary entrained values had little change during FR (Figure 1).

The sleep stage characteristics for all subjects were compared as a function of sequential experimental nights during the three experimental conditions (Entrained, Free-running, and Re-entrained). The values of REM% of TST were remarkably constant throughout and did not differ significantly as a function of experimental conditions (Table 1). The Stages 3+4% of TST did increase to a small extent from the entrained (27.8%) to the FR (29.8%) condition, especially during the last 6 FR sleep periods (Table 1). A small average increment occurred during the five re-entrainment nights (32.2%) for 5 subjects.

An interesting result was obtained when comparisons were made for REM% of TST, by subject and by experimental condition (Table 1). There was considerable variability in REM sleep across subjects (range 15 to 30%), during the entrained period. However, the intra-subject variability was very small as a function of experimental conditions. This was not the case for Stages 3+4 since both the inter- and intra-subject variability was similar in all 3 experimental conditions. These results indicate that each subject maintained an individual control of REM% of sleep time which was independent of the entrained or free-running state. This does not appear to be the case for Stages 3 and 4 sleep.

Three subjects (FR 3, 4, & 10) consistently had long sleep periods associated with long sleep-wake cycle lengths. There were a total of 26 sleep periods lasting 12 hours or longer. These long sleep periods differed from the short sleep periods. The timing of the onset of these long sleep periods occurred at a different phase of the subjects circadian temperature rhythm(130 degrees to 270 degrees, 0 degrees = mid-trough) than the onset of the short sleep periods (270 degrees to 120 degrees). In addition, during the long sleep episodes, sustained Stages 3 and 4 sleep would characteristically occur between 12 and 18 hours after sleep onset. However, the first 4 hours of the long sleep periods did not differ significantly in regard to the characteristic timing and amount of Stages 3 and 4 sleep seen under entrained conditions. Thus, despite normal amounts of 3-4 sleep present at the onset of these long sleep periods, Stage 3-4 would reappear after 12 to 16 hours of sustained sleep (Figure 2). Although occasional awake episodes interrupted these long sleep times, (especially for subject FR 10) they were not sufficiently long to explain the reoccurrence of Stages 3 and 4.

Another characteristic difference between the long and short sleep periods was the timing and amount of REM sleep within the first 3 hours after sleep onset (Table 2, Table 3). All of the sleep periods which had a very small REM latency (20 minutes) were short sleep periods during the FR conditions. The mean REM latency (sleep onset to onset of first REM period) clearly decreased for 9 of the 10 subjects(FR 9 was the exception) comparing entrained to the free-running condition. A partial recovery took place during the re-entrainment conditions. In addition, the mean total minutes of REM sleep in the first 3 hours of sleep increased for 8 of the 9 subjects (subject FR 9 excepted) between entrainment and free-running. However, during re-entrainment

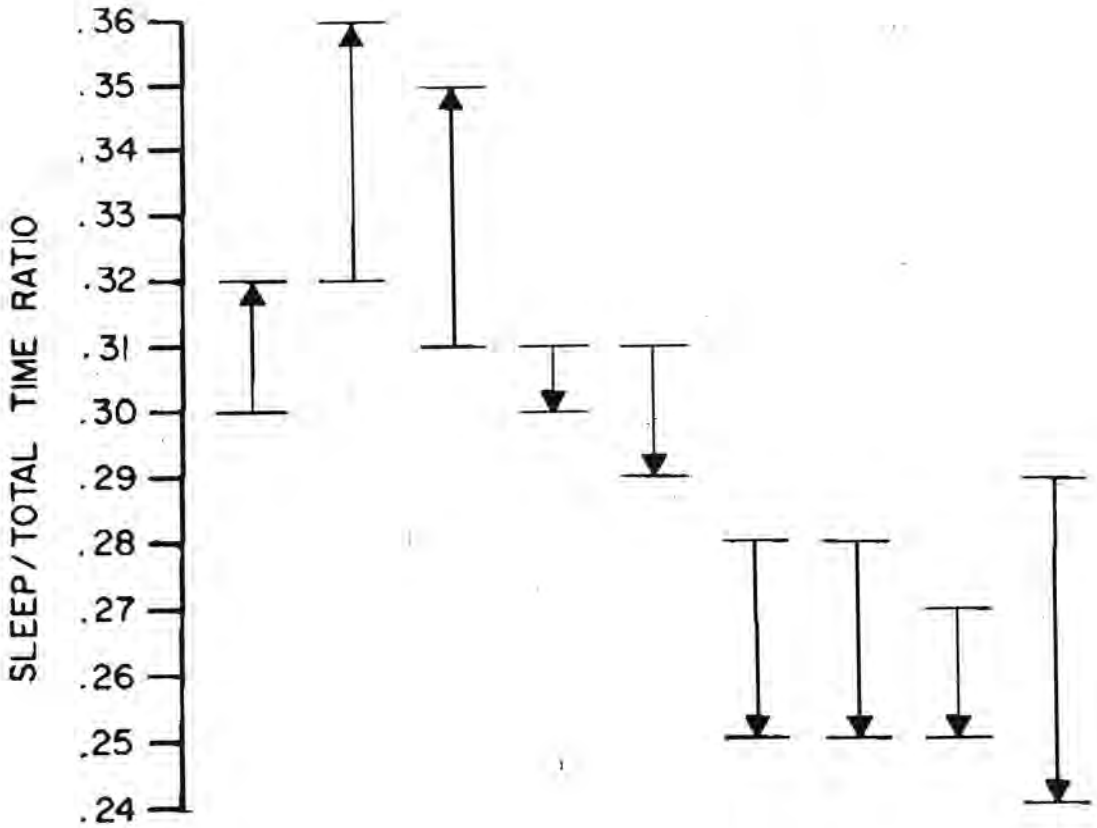

Figure 1. Sleep to total time ratio for each of the 9 subjects. The arrows indicate the change from baseline entrained to the Free-running condition.

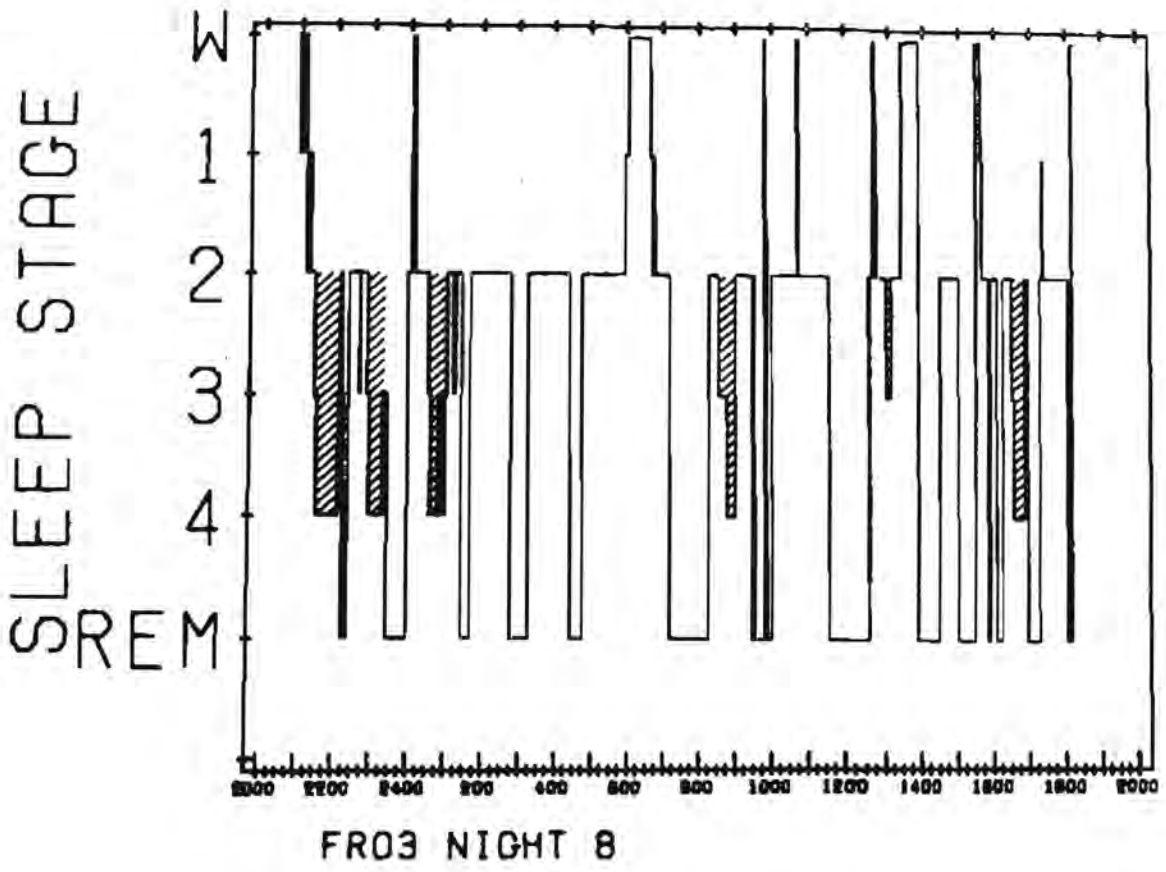

Figure 2. Reappearance of Stages 3 and 4 sleep during a long sleep period (greater than 21 hours) in subject FR 3 on Free-running Sleep period 8.

Table 2

REM Latency (Mins) and REM Time (Mins) During First Three Hours After Sleep Onset of Subjects During Experimental Conditions

Entrained	FR 1	FR 2	FR 3	FR 4	FR 5	FR 6	FR 7	FR 9	FR 10
REM Lat.	78	127	95	94	79	109	72	52	66
REM Mins	15	21	15	7	15	7	13	23	11
Free-running									
REM Lat.	59	70	73	64	57	87	61	56	52
REM Mins	43	37	24	15	36	28	28	23	18
Re-entrained									
REM Lat.				79	72	90	74	64	
REM Mins				14	26	23	34	19	

Table 3

Mean REM Latency and Sleep Period Duration During Free-Running for Four Subjects

	Long Sleep Periods			Short Sleep Periods		
Subject	N	REM Latency	Sleep Duration	N	REM Latency	Sleep Duration
FR 3	4	73.3	1071	3	72.0	591
FR 4	7	67.0	867	2	53.0	637
FR 10	7	65.7	816	5	31.6	406
PR 1	7	57.2	850	6	47.0	491

these values did not return to baseline. The timing and amount of REM sleep during the first 3 hours after sleep onset in PR 1 was determined during the free-running condition when he had the alternating long and short sleep-wake cycles. We determined the mean total sleep duration on sleep periods with short (< 30 minutes) and long (> 30 minutes) REM latency and on sleep periods with REM amounts greater or less than 30 minutes during the first 3 hours

following sleep onset (Table 4). We found a statistically significant difference ($p < .025$) with the longer sleep periods associated with less REM sleep and the shorter sleep periods associated with more REM sleep during the first 180 minutes after sleep onset. A similar difference was also found for REM latency but this was not statistically significant ($p < .2$). In addition, all the nights with a short REM latency (< 10 minutes) and the nights with more than 30 minutes of REM sleep in the first 3 hours except one occurred within 90 degrees of the nadir (0 degrees) of the circadian temperature rhythm. In addition, for 12 REM onsets which occurred within 10 minutes of sleep onset, 11 occurred within 60 degrees of a specific phase (mid-trough) of the circadian temperature rhythm.

Table 4

Relationship of Total Sleep Duration to REM Latency and REM Amounts During the First 180 Minutes Following Sleep Onset for 67 Circadian Days of "Free-Running" Sleep-Wake Cycling During Temporal Isolation in Subject PR 1

	REM Latency		REM Amount During First 180 Minutes	
	< 30 Mins	> 30 Mins	< 30 Mins	> 30 Mins
Number of Sleep Periods	27	41	40	28
Mean Amount (Mins ± S.D.)	8.2±6.6	74.2±13.0	20.1±6.0	45.7±10.9
Mean Total Sleep Duration (Mins ± S.D.)	500±128 ←**	→547±138	555±136 ← *	→480±121

* $p < .025$ ("T" Test)
** $p < .20$ ("T" Test) Not Significant

An analysis was made of REM-non-REM sleep cycling during the different experimental conditions. The latency in minutes from sleep onset to first mid-REM period, first mid-REM to second mid-REM, etc., was determined. It was found that except for a shortened latency from sleep onset to the mid-first REM period during free-running there were no differences in cycle lengths as a function of experimental condition. There was a consistent decrease in cycle length for the fourth and fifth cycle for each condition. The sleep cycle length remained stable ($\bar{x}$ 85 minutes) for up to 11 cycles during the long sleep periods (> 10 hours). Thus, there is no evidence that sleep stage cycle length is altered by the increased sleep-wake period length during free-running conditions. In addition, previous reported results (Feinberg, 1974) of a stable but slightly reduced cycle length when sleep is extended, are confirmed by these data for those long sleep periods which extend from 10 to 20 hours.

Body Temperature Rhythm

The mean core (rectal) temperature for all subjects as a group was essentially the same in all three conditions. However, for each subject in Group A there was an increase in the mean temperature during FR whereas there was a decrease for each subject in Group B. During re-entrainment, the mean value of most subjects had returned to that of the entrained section.

During the entrained condition, the rectal temperature curve (values obtained every minute) demonstrated the well described sharp fall (1-2 degrees F) following sleep onset (Aschoff, 1970; Aschoff et al., 1976; Timball, Colin, Boutelier, & Guieu, 1972). A small decrease in temperature typically occurred at approximately 3 hours before sleep onset with a sharp elevation of temperature at the end of the sleep period. During "Free-Running" for all subjects in Group A a change in both phase and shape of the curve occurred (Wever, 1973) (Figure 3). The temperature began to decrease 6 to 8 hours prior to sleep onset. At the time of choosing sleep the body temperature was close to the lowest value of the circadian rhythm. An additional small fall of temperature (0.5 degrees F) took place just after sleep onset during FR. During re-entrainment, the curve was similar to that found in the entrained condition, although it had not fully established the original shape. In two subjects with long sleep periods a wave shape pattern was educed during the FR condition at the same period length as the sleep-wake cycle (39.1 h (FR 3) and 37.6 h (FR 4)) and one at a period length near 25 hours (24.6 h (FR 3) and 24.7 h (FR 4)) (Figure 4). The curves at the long period lengths, resembled those in the entrained conditions (normalized to 360 degrees), both in shape and phase relationship to the average sleep time. These results suggest that the approximately 40 hour component in the temperature rhythm was a "response" to sleep onset in the sleep-wake cycle rather than an independent self-sustained rhythm. In each of these cases (FR 3, 4, 10) as mentioned above there was also an approximately 25 hour component in the circadian temperature rhythm. The amplitude was small (approx. 1 degree F) compared to the entrained condition (approx. 2 degress F) and compared to subjects FR with a sleep-wake cycle of approximately 25 hours (1.5 to 2.0 degrees F).

Subject PR 1 had a small drop of overall mean temperature in the entrained compared with the Free-Running Condition (98.42 degrees to 98.17 degrees F). He had a circadian temperature period length of 25.0 hours during the first 30 free-running days which shortened to 24.55 during the next 50 days.

Plasma Cortisol Pattern

We have been successful in obtaining plasma samples at 20 minute intervals for each of 9 subjects during the experimental conditions (FR 1, FR 10; total samples obtained, 15,000).

During the entrained condition all subjects demonstrated the normal episodic pattern of secretion during each 24 hour period. The typical pattern was evident with very low values just prior to and during the first 3 hours of sleep, followed by a series of secretory episodes during the latter half of the night (Figure 5). An intermittent, episodic secretory pattern was present during the waking day (Weitzman et al., 1966; Weitzman et al., 1971). The educed wave form for the entrained condition also demonstrated this circadian

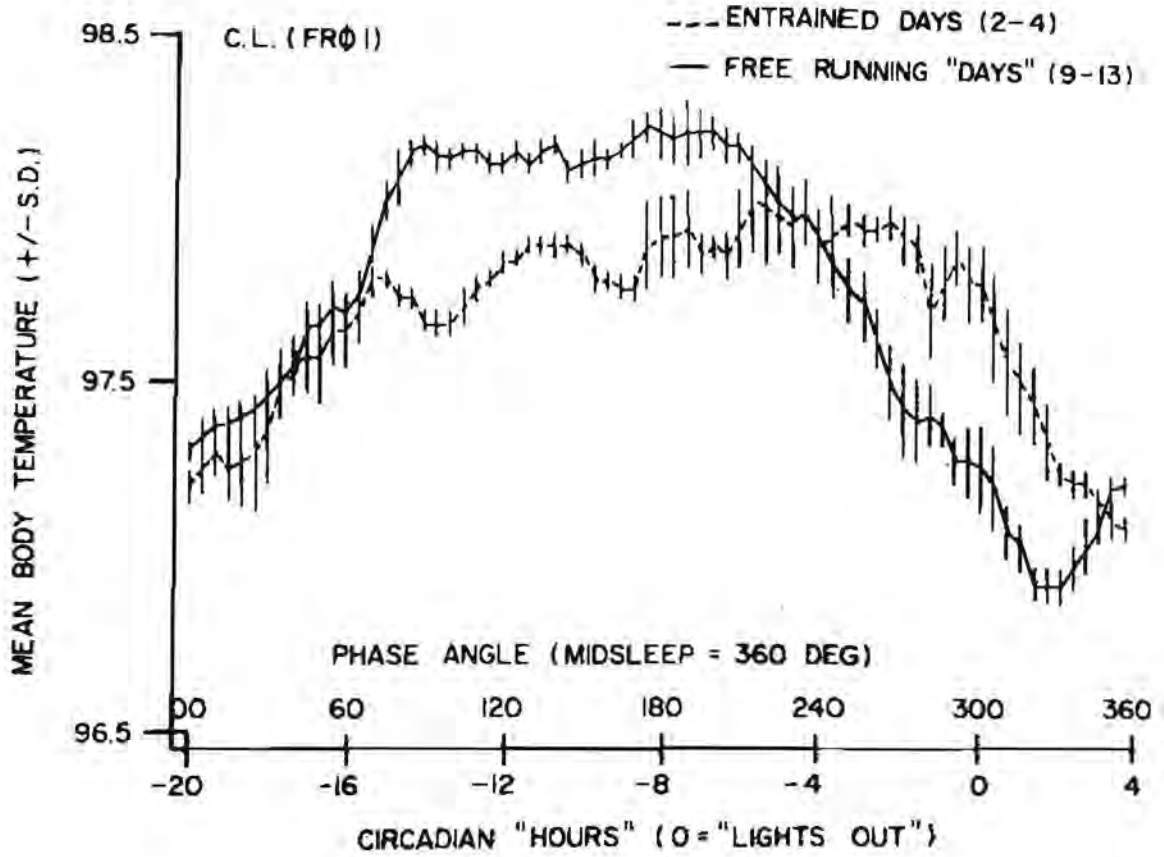

Figure 3. Educed wave form and S.E. of core temperature for subject FR 1 during the entrained and Free-running condition. The mean sleep time during the Free-running condition was 483 minutes (see Table 1). Lights out was at 0 circadian hours and the mid-sleep time was at a phase angle of 360 degrees.

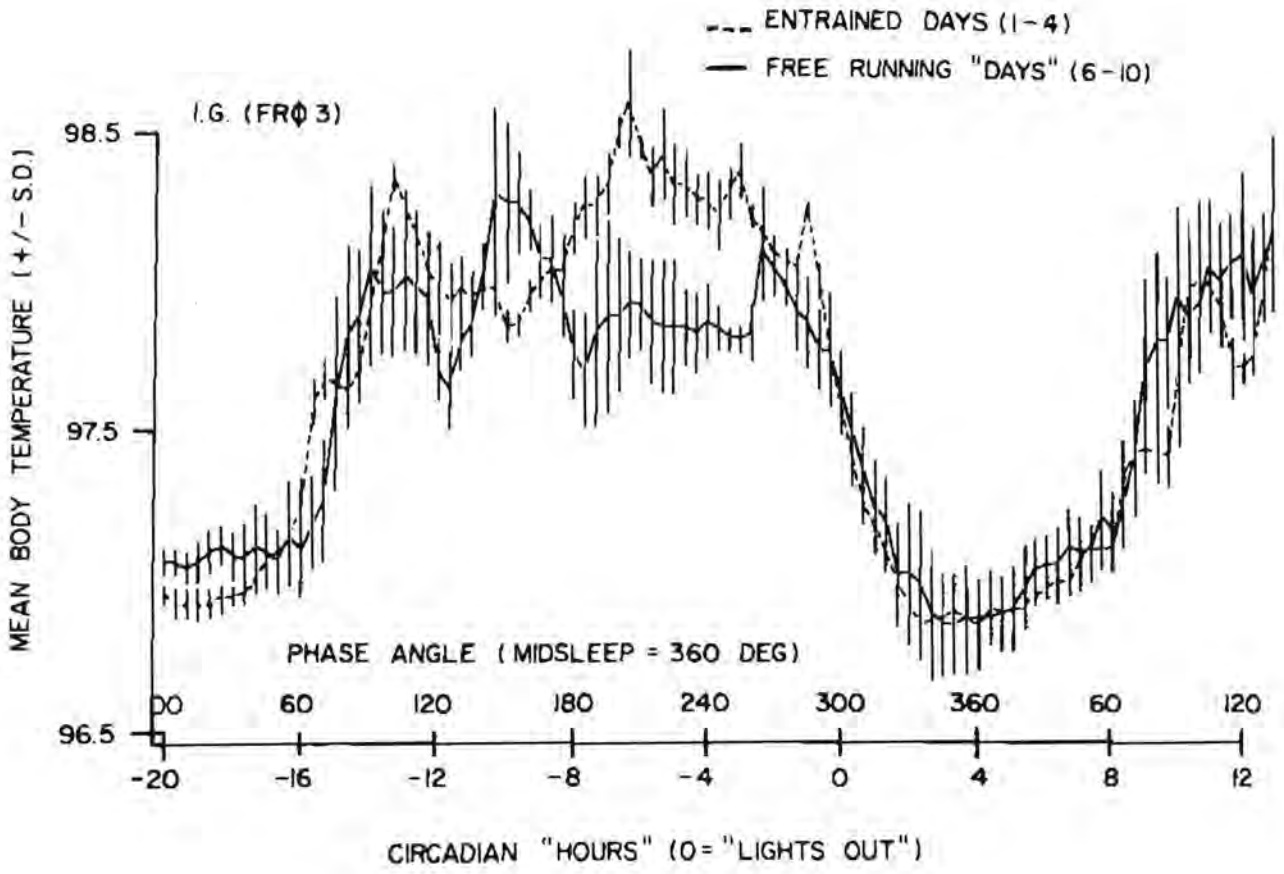

Figure 4. Educed wave form and S.E. of core temperature for subject FR 3 during the entrained and Free-running conditions. The mean sleep time during the Free-running condition was 830 minutes (see Table 1). Lights out was at 0 circadian hours and the mid-sleep time was at a phase angle of 360 degrees.

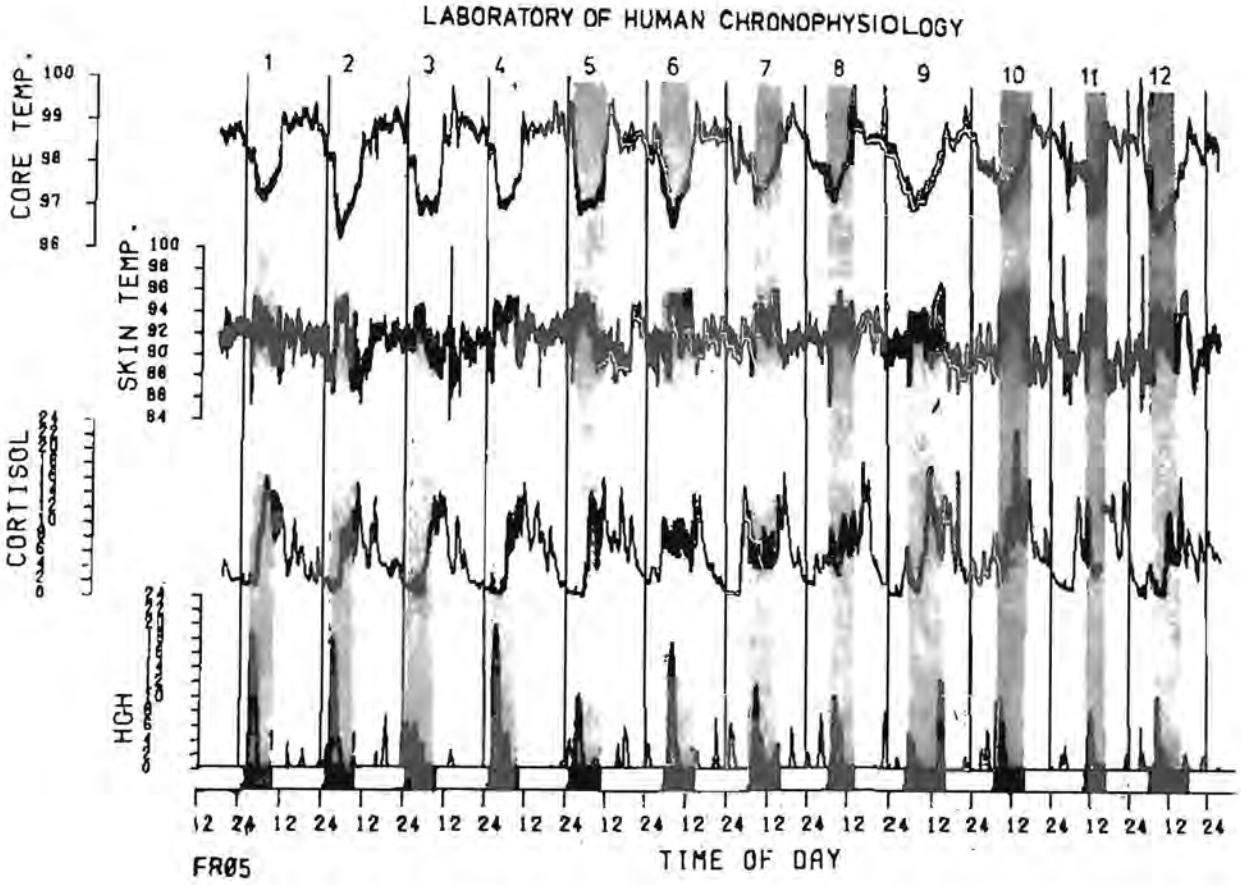

Figure 5. Continuous plot of core and skin temperature (F°), plasma cortisol (µg/100ml) and growth hormone (ng/ml) for subject FR 5 during the entrained (days 1-5) and the Free-running (days 6-12) condition. The shaded areas are the lights out sleep time.

pattern of hormonal activity. During the free-running condition, a clear phase advance and change of wave shape of the circadian cortisol curve was evident for subjects FR 5, FR 6, FR 7 (Figure 6). The nadir of the curve was now occurring 100 to 150 degrees in advance of that during entrainment with respect to sleep onset. In addition, the average rate of rise of cortisol after the low point was much more gradual, nevertheless reaching the highest value at approximately the same time, namely the end of the sleep period.

It is important to emphasize that the process of wave form eduction produces an overall mean curve at a defined period length and therefore will "smooth out" specific point related events. Examination of the cortisol time series itself revealed that on many "free-running" days, especially with a progressive phase delay, cortisol would be secreted just before sleep onset and then would stop being secreted for several hours just after sleep onset.

The duration of this inhibition was 1-3 hours at the beginning of sleep and did not continue throughout sleep. Sleep onset was therefore used as a "zero" point about which a time locked response cortisol curve was obtained in several subjects. All demonstrated a clear pattern of cortisol inhibition following sleep onset. Therefore during the free-running condition a phase advance of cortisol occurred in relation to the sleep period, the overall wave shape was changed and a specific sleep related inhibition of cortisol secretion was apparent. During the re-entrainment condition, a similar pattern was evident since the phase relationship between the cortisol rhythm and sleep had not yet returned to normal. Therefore the subject was going to sleep when the concentration of the hormone was high. Evidence that this sleep related inhibition may well be operative even in subjects habitually living on a 24 hour routine may be deduced from the data obtained during the transition from the entrained to the free-running condition on those nights when a phase delay of sleep onset on a single night exceeded 2-3 hours. On those occasions, the hormone was released just before sleep and then immediately inhibited after sleep onset.

It thus appears that the episodic pattern of cortisol secretion is influenced both by an endogenous rhythmic component, not directly related to the behavioral sleep-wake cycle and a specific sleep (or lights out in bed) related component. Whether other daily behavioral events such as sleep onset, lights on, out of bed, meal time, etc., are also determinants of the episodic pattern will require further detailed analysis of the extensive data we have obtained in these studies.

Growth Hormone Pattern

HGH was found to be secreted in an episodic normal manner in all subjects with the typical pattern of brief episodes of secretion (1-2 hours) followed by long inter-episode intervals (6-12 hours) with no HGH detectable (Weitzman & Hellman, 1974) (Figure 5). The hormonal concentration was less than the overall average (1 ng/ml) 80% of the time. A striking highly consistent relationship between sleep onset and an episode of HGH secretion was found for all three experimental conditions for all subjects (Sassin, Parker, Mace, Gotlin, Johnson, & Rossman, 1969; Weitzman et al., 1975). A clear secretory episode followed sleep onset approximately 90% of the time. Thus far no independent rhythm of HGH could be detected but further analysis for an ultradian, or specific behavioral related event will be searched for.

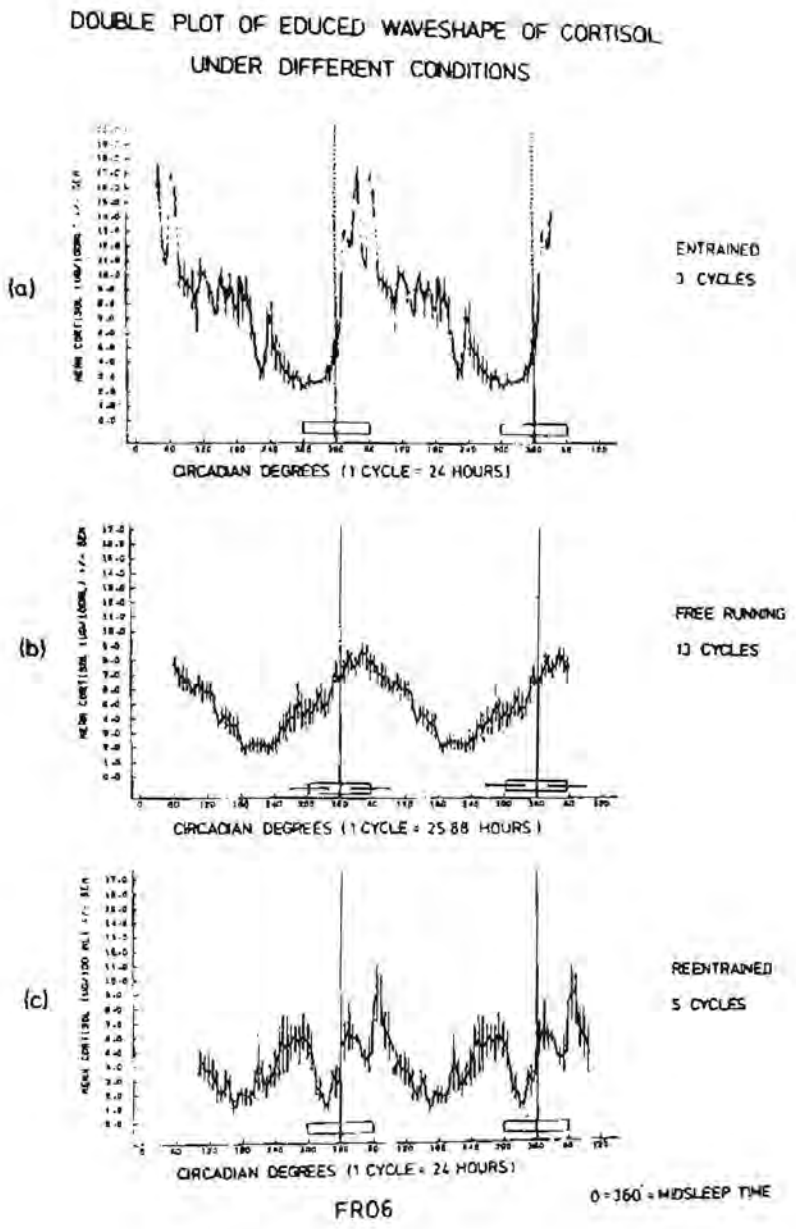

Figure 6. Double plot of educed wave shape of plasma cortisol concentration for subject FR 6 during entrained, Free-running and re-entrained condition. The horizontal bar and vertical dotted line represent lights out sleep time and mid-sleep time respectively.

Summary and Conclusions

We confirm previous studies that biological rhythms of human beings free-run at period lengths greater than 24 hours, typically at approximately 25 hours, but with individual variability. After a variable time of free-running, many normal humans will spontaneously develop "long" biologic days (35 hours) and often these will alternate with "short" days (approx. 25 hours).

During free-running, although the sleep to total time ratio remains remarkably constant (approx. .30), short sleep periods (10 hours) occur at a specific phase angle of an internal circadian rhythm (e.g., body temperature) whereas long sleep periods (12 hours) take place approximately 180 degrees out of phase with the short sleep periods, but maintain the same period length. Sleep stage organization changes during "free-running" such that REM sleep advances to an earlier time during sleep, with a shortened REM latency (occurring at times less than 10 minutes after sleep onset) and increased amounts during the first 3 hours of sleep. The total REM amount and percent for the entire sleep period, however, remains constant. The timing and amount of REM sleep following sleep onset also occurred preferentially at a specific phase of the circadian temperature cycle, strongly supporting the concept that certain sleep processes in the brain are endogenous biological rhythms. The Stage 3-4 sleep distribution remains essentially the same during the three experimental conditions. During the long sleep periods (12 hours), Stages 3 and 4 recur following 14 to 16 hours of sleep indicating that these stages are not dependent on length of prior waking but may be related to length of prior elapsed time.

The core (rectal) temperature develops an approximate 25 hour rhythm in humans during free-running, but the wave-shape changes such that a phase advance (6-8 hours) of the falling phase develops in relation to the onset of sleep. The subject usually then selects sleep when the circadian temperature approaches its lowest value of the day. In addition, at the time of sleep onset (lights out and in bed) there is an additional drop of body temperature. This is especially noted when sleep onset occurs when the immediately preceding core temperature is high (e.g., for the long sleep periods).

Measurements of plasma cortisol throughout each study demonstrated two components of the circadian cortisol curve during free-running. One component had a phase advance (6-8 hours) relative to sleep onset whereas a second component clearly followed sleep onset. This second component appeared to be a sharp inhibition of cortisol secretion during the first 2-3 hours of sleep interrupting a rising phase of the hormonal curve. Growth hormone secretion, on the other hand, was intimately related to the first 2 hours after sleep onset. A sharp episode of hormonal secretion occurred just after sleep onset for almost all sleep periods. No other independent circadian rhythm of GH has been detected thus far.

These and previous reported studies emphasize the lawfulness of biological rhythm functions in man and demonstrate the importance of the methodology using temporal isolation and the analysis of "free-running" rhythms to unravel these chronobiological processes.

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Supported in part by Grants NIA-AG #00792 and NIMH #28460 of the U.S. Public Health Service.

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July 1981

DHHS (NIOSH) Publication No. 81-127