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Endocrine Rhythms across Sleep–Wake Cycles in Normal Young Men under Basal State Conditions

Donal C. Parker, Lawrence G. Rossman, Daniel F. Kripke, Jerome M. Hershman, William Gibson, Christine Davis, Kevin Wilson, and Eugene Pekary

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I. INTRODUCTION

Recognition of rhythmical influences upon man are evident in his earliest myths. His daily sleep–wake cycle, which was a magical and restorative gift from the gods, and his environment's daily light–dark cycle, which he subsequently had Prometheus steal from the gods to further research into control mechanisms of fate, are recurrent symbols for man's life, death, and renascence.

Far less tragic and interesting concepts of sleep have since intervened. Sleep has been pejoratively seen as a time of lack of consciousness, purposeful behavior, activity, or feeding, of lessened metabolism and temperature or of unstressed unchallenged homeostasis. Such is not the "stuff that dreams are made on." The Freudian hope of an Orphic path to the nether world of the unconscious was the next brief candle lit in sleep. Subsequent recognition of intense degrees of neural activation and synchronization that occur in sleep and of the objective equivalent of dreaming, the rapid eye movements or REMs (Dement and Kleitman, 1957; Loomis *et al.*, 1937), resurrected 20th century interest in sleep. Like all rhythmical events in the fortunes of man, this brief history of sleep comes full circle to the subject of the present chapter and the current acrophase of man's interest in sleep. Here we will present evidence of nychtohemeral (night > day) rhythmicity in man's hormones that subserve growth, maturation, reproduction, nurture, energy regulation, mineral metabolism, adaption, and survival. Thus, these hormones seem to be an existential and physiologic subset of "fate." These hormonal maxima occur in relation to man's legendary cycles of sleeping and waking and of light and darkness in which they represent daily periods of rather intense hormonal activation.

It is now well accepted that the erstwhile Zeus of glands, the pituitary, is complexly regulated by man's central nervous system through a series of still largely unknown neural inputs and oscillators that are afferent to known hypothalamic neurotransmitters and hypophysiotropins. These latter are the latest generation of hormones, the specific releasing and/or release-inhibiting factors for each tropin of the anterior pituitary (Burgus *et al.*, 1969; Brazeau *et al.*, 1973; Baba *et al.*, 1971). In response to such stimulation, the pituitary tropocyte promptly releases its specific and short-lived peptide hormone into the venous blood, where concentration fluxes can then be determined by sensitive specific radioimmunoassays (Berson and Yalow, 1973). The signals that induce hormone release may be generalized as environmental transients that may be single (e.g., test agents, stress) or rhythmically repeated, or as endogen-

ous signals that may also originate singly (e.g., in hypoglycemia, fever, exercise) or be rhythmically repeated (e.g., sleep, activity, feeding, light perception, or simply the passage of time).

II. HORMONAL RELEASE PATTERNS

The release patterns into plasma for each pituitary hormone may be generalized into several forms.

1. Daily, diurnal, nychtohemeral, or circadian variation. These loose synonyms indicate a pattern of a single maximum and minimum per day, e.g., a slow frequency of one cycle/day or a period of oscillation of about 24 hours. It is this frequency that we will deal with in this chapter. Daily means simply that; nychtohemeral indicates the maxima to be nocturnal; diurnal or hemeronychtal indicates the maxima to be in daytime. Circadian restrictively imputes the variation's persistence at a period near 24 hours in the absence of environmental synchronizers or time cues as well as the rhythm's capacity to be synchronized to a precise 24-hour period by such a synchronizer. It thereby also infers an endogenous basis for the hormonal variation. Thus we have preferred to use the term nychtohemeral rhythmicity for the repetitive daily patterns in the current absence of such information about most hormones other than cortisol (Krieger and Aschoff, in press).
2. Briefly episodic release: All hormones exhibit many short episodes of variation across the day. Briefly episodic is simply descriptive and allows no imputation about rhythmicity, waveform, or origin. Other terms that have been used for this kind of release have been oscillatory, which implies a sinusoidal waveform; pulsatile, which imputes a nonsinusoidal waveform that is usually a rapid up and slower down stroke and that is thought to be characteristic of a relaxation oscillator; circhoral, which means a duration of about an hour; or ultradian, which imputes rhythmicity with a frequency of more than one cycle per day (e.g., period of < 20 hours). We will not deal further with briefly episodic release here, other than to call attention to the fact that it is obscured by our technique of averaging across subjects by clocktime.
3. Very slow or infradian frequencies (less than one cycle per day), such as menstrual or seasonal, also are not considered here though we have indicated the month of the year in which studies were done (Table I) and recommend that others do likewise as a first step in this direction.

III. PURPOSE OF PRESENT STUDIES

Our long-term goals have been to define normal patterns of daily hormone release, to learn how they originate and are physiologically modulated, and then to use this information to understand pathophysiology and apply it to endocrine diagnosis and treatment. Our present purpose is firstly to present normative data of hormonal variation across basal sleep-wake cycles of healthy young adult males that demonstrates nychtohemeral rhythmicity both visually and analytically and secondly to demonstrate the influence of sleep upon such rhythmicity.

IV. BASAL STATE: STUDY CONDITIONS AND METHODS

We have studied 33 healthy nonobese young adult men between the ages of 19 and 30 under carefully defined conditions that represented a basal state that was free from stressful transients but that was representative of their usual everyday life in regard to its schedules. An attempt to fix the sleep-wake cycle was made, and the integrity of the sleep phase was objectively monitored polygraphically. Samples were drawn frequently so that maxima would not be missed in these short-lived hormones and so that acrophases would not be misrepresented. A regular undisrupted life schedule of sleeping, eating, and waking at usual times and of abstinence from use of psychoactive drugs was prerequisite; and all subjects were accommodated to the environs and study techniques prior to onset of sampling. In all studies, exposure to the natural light: dark (L:D) cycle and usual social cues were maintained. Awareness of 24-hour time cues was maintained by radio and TV programming, watches, clocks, and visiting schedules. Bedtime and artificial illuminative "lights out" were held to 22:30–23:00; awakening and "lights on" held to 06:30–07:00. If they spontaneously awakened earlier they remained in bed until 06:30. Naps or lying supine in bed were not allowed during wakeful hours. No exercise stresses were permitted as they maintained sedentary activity in wakefulness. Meals were eaten at 07:30–08:00, 12:30–13:00, and 17:30–18:00, and water or noncaloric beverages were permitted across the day ad lib. Under local anesthesia, indwelling venous catheters were inserted into forearm veins and carefully secured and dressed. A dilute (5 u/cc) heparin-lock system that included a small-bore 10-ft extension line (1.8 ml void volume) allowed sampling from outside the bedroom. Blood samples of 2 ml were drawn every 20 minutes across

nique was used to obtain the "raw" hormonal plots for GH (Fig. 1), for PRL (Fig. 2), for TSH (Fig. 3), for LH (Fig. 4), and for testerone (Fig. 5). In Fig. 6, cortisol and body temperature, two known circadianally rhythmic functions are shown as reference plots. The mean hormone concentrations during the first 24 hours in Table II are equivalent to the 100% ordinate value of these figures.

1. Growth Hormone

The daily maximum is seen to occur repetitively each night at 24-hour intervals—e.g., the raw data evidence nychtohemeral rhythmicity as well as nychtohemeral variation under our basal state study conditions (Parker *et al*, 1979b; Parker and Rossman, 1973, 1974). The onset of rise, peak, and decline of these nightly GH peaks are seen to be restricted to the first half of the sleep interval. However, note the shape of the nightly maxima's waveforms for GH. Their durations above the

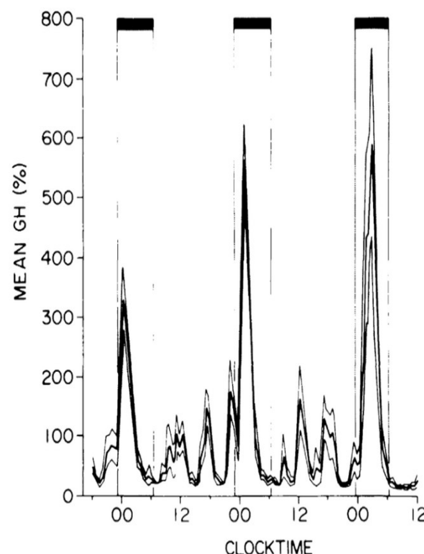

Fig. 1. Mean percentage of GH concentration (± SE) at 20-minute intervals across basal sleep-wake cycles of normal young men. The data from 26 studies of 24–48-hour duration in 21 healthy young adult male subjects were pooled for averaging at 20-minute intervals across clocktime. Gray-black bar represents sleep. Original GH concentrations were converted to percentage of appropriate 24-hour mean before averaging to "normalize" subjects' release patterns. Since some studies began in the morning, 3 nights of sleep are seen. See text for description of basal state conditions and Tables I–II for further details about studies.

100% level of about 4 hours are too short for a cosine whose period is 24 hours. Similarly, it is evident that the nightly maxima are not the only GH peaks that remain aligned in clocktime across subjects but that at least three or four smaller episodes of release occur in wakefulness: near awakening, late in the morning, late in the afternoon, and in the evening before sleep begins. These latter aligned peaks clearly indicate the presence of shorter (ultradian) periodicities in GH release across the wakeful day and predict that a complex band, rather than a single frequency, characterizes the 24-hour GH patterns under our conditions. These daytime peaks were recognized shortly after the development of GH RIA in 1963 (Glick *et al.*, 1963) and have been called postcibal or postprandial peaks that were attributed to the earlier rise and fall in glucose and insulin that followed feeding (Glick *et al.*, 1965; Roth *et al.*, 1963). However, we have shown that similar but smaller rises still occur at these times when meals have been skipped (Parker and Rossman, 1973). The peaks occur at similar intervals in fasting (Parker *et al.*, 1979a,b) or during intravenous glucose pulsing (Parker *et al.*, 1979b). In this latter instance, the peak occurs without relation to the period of the glucose pulses. Thus, rather than a direct cause–effect relationship of periodic feeding to wakeful GH peaks, feeding may act more as a suppressive modulatory or masking influence which then wanes or is escaped from as endogenous GH release resumes after feeding. This would also perhaps explain some of the variability in the meal-postcibal peak intervals that is often seen. A more detailed description of the multifrequency pattern of GH across the 24 hours can be found in Parker *et al.* (1979b), where autocorrelative, variance spectra, and periodographic techniques were employed in their analysis.

2. Prolactin

In Fig. 2, the daily maxima are again seen to recur rhythmically each night under our basal state conditions (Parker *et al.*, 1973). The peak is again almost entirely restricted to the sleep interval. The sleep peaks of PRL are not coincident in time with those of GH (Sassin *et al.*, 1972). If the locus of the sleep phase is not carefully fixed by objective monitoring, greater spread in clocktime alignment of peaks and more difficulty in demonstrating rhythmicity occurs. In contrast to the GH pattern, the PRL peak in sleep in Fig. 2 appears to be the only major aligned episode of release across the 24 hours. Like GH, PRL's peak durations above 100% of about 7 hours are not characteristic of a cosine of a period of 24 hours but are less markedly different from this ideal circadian cosine than are those of GH's.

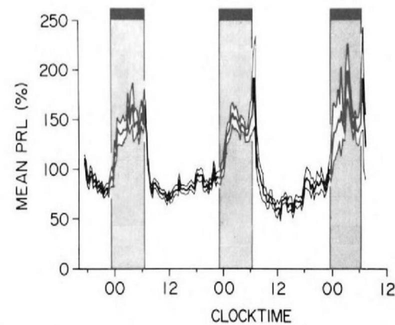

Fig. 2. Mean percentage of PRL concentration (± SE) at 20-minute intervals across basal sleep-wake cycles of normal young men. Data from 25 studies of 24-48-hour duration in 20 subjects. Details as in Fig. 1.

3. *Thyrotropin*

The daily maxima are seen to rhythmically recur each evening and to precede the onset of the sleep interval (Parker *et al.*, 1976; Azukizawa *et al.*, 1976; Weeke, 1973; Weeke *et al.*, 1975; Alford *et al.*, 1973). The rise to peak begins in the early evening and then declines occur across sleep. These results contrast with previous reports in which the sleep phase was not carefully fixed or objectively measured and in which the loci of the maxima were found to vary across the entire night or to not be consis-

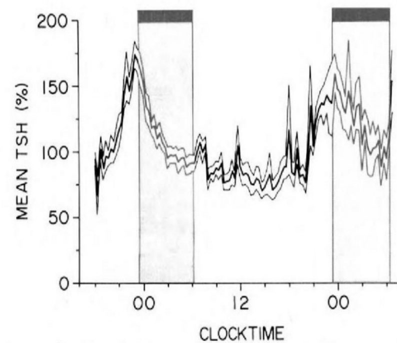

Fig. 3. Mean percentage of TSH concentration (± SE) at 20-minute intervals across basal sleep-wake cycles of normal young men. Data from 15 studies across 24-48 hours in 12 subjects. This figure does not include data beyond subject 23 of Table IV. Details as in Fig. 1.

tently observed (Webster *et al.*, 1972; Nicoloff *et al.*, 1970; Vanhaelst *et al.*, 1972; Van Cauter *et al.*, 1974, 1975). This contrast indicates that sleep is almost as important a determinant of the locus of maxima of this TSH rhythm as it was for hGH and PRL (Parker *et al.*, 1976).

4. *Luteinizing Hormone*

This gonadotropin, which drives male testosterone production by the testes' Leydig cells, has been shown to exhibit nychtohemeral and sleep-related rhythmicity during masculine puberty (Boyar *et al.*, 1972; Parker *et al.*, 1975; Parker and Rossman, 1974; Judd *et al.*, 1974b; Kapen *et al.*, 1974). Indeed, such LH rises in sleep have been shown to initiate puberty as they are followed momentarily by rises in plasma testosterone concentrations (Judd *et al.*, 1974b; Parker *et al.*, 1976; Boyar *et al.*, 1974), which induce the changes in secondary sexual characteristics used to assess and stage male puberty. These sleep-related changes in LH and T have been shown to precede the first recognizable changes of Stage 2 puberty (Judd *et al.*, 1977). As boys progress across puberty, they have been shown to acquire enhanced episodic release of LH and T in wakefulness until in the final pubertal Stage 5, the adult pattern of equivalent pulse height of LH release across the sleep-wake cycle occurs (Parker *et al.*, 1975).

The consensus from studies with frequent sampling is that adult males do not exhibit sleep-wake or nychtohemeral variation in LH concentrations (Boyar *et al.*, 1972; Krieger *et al.*, 1972; Yen *et al.*, 1974). That a nychtohemeral variation in T persists in the adult male is now also the consensus (Judd *et al.*, 1974a; Southren and Gordon, 1975; Baker *et al.*, 1975). However, the LH plot in Fig. 4 seems to exhibit the presence of an aligned but low amplitude rise in LH across the night in these 16 studies. This suggests that nychtohemeral rhythmicity in LH may exist in young men under our basal state conditions. This may represent the case only for recently post-pubertal young men such as those in our study, or it may have been overlooked previously because of its low amplitude and the insensitivity of static or linear tests of significance. Aschoff (1979) in a review of others' data, which he has referenced to estimated mid-sleep and replotted, has recently arrived at the similar conclusion that "further search for a circadian component underlying the rhythm of plasma LH concentration seems justified" (Aschoff, 1979). That LH rhythmicity as seen in Fig. 4 may not be solely attributable just to the present careful fixation of the sleep phase in our studies is suggested by the duration (ca. 9 hours) of the nocturnal elevation above 100% that extends beyond both ends of the sleep interval in Fig. 4.

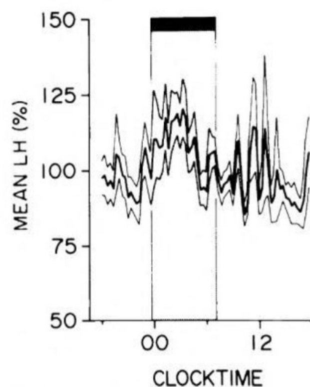

Fig. 4. Mean percentage of LH concentration (± SE) at 20-minute intervals across basal sleep–wake cycles of normal young men. Data from 16 studies of 24–48-hour duration in 13 subjects. Details as in Fig. 1.

5. *Testosterone*

In Fig. 5, the nocturnal peak in T is more apparent and less equivocal than that of LH. This is attributable to its larger amplitude, lesser variance, and longer duration. The individual maxima usually occur near the end of sleep. However, the onset of mean rise to peak and the decline to below 100% both reside well beyond the confines of sleep as the >100% interval lasts ca. 14 hours. Thus T comes closer than any of the previous hormones to exhibiting a cosine waveform. However, T plots from an individual subject do not show this unimodal pattern as

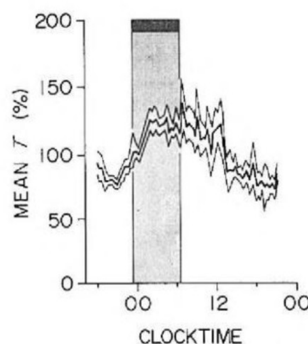

Fig. 5. Mean percentage of T concentration (± SE) at 20-minute intervals across basal sleep–wake cycles of normal young men. Data from 10 studies of 12–24-hour duration in seven subjects. Details as in Fig. 1.

clearly since release tends to occur in two or more large and slow (ca. 3–5 hours long) peaks/day. The largest and the most consistent of these occurs across the latter half of sleep and accounts for the mean pattern seen in Fig. 5.

6. *Cortisol*

Cortisol (Krieger and Aschoff, in press) and body core temperature (Fig. 6) have well established human circadian rhythms and are often used as phase reference points for other rhythms (Aschoff, 1979). Here under our basal conditions these well-known patterns are replicated. The cortisol maximum occurs at the end of sleep rather than later in the morning as often reported in previous studies, and its nadir segment in the early sleep interval shows an interesting reduction in variance compared to the rest of the day. This latter suggests that a sleep-related inhibition of secretion may occur. Because body temperature falls in sleep regardless of its location across the day (Aschoff, 1979), this waveform may also not be a purely or simply expressed circadian event. Neither cortisol or temperature exhibits a true daily cosine waveform as the >100% segment of the corisol peak lasts only about 7 hours and the <100% segment of the temperature is only about 8 hours.

Thus, our raw hormonal data evidence nychtohemeral variation and rhythmicity in growth hormone, cortisol, prolactin, testosterone, thyrotropin, and probably luteinizing hormone when ranked by amplitude of the daily maxima in Figs. 1–6 and given in Table VI. When listed by sequential order in clocktime of maxima across the night, the order is presleep TSH, early sleep GH, mid-late sleep PRL and LH, and end-

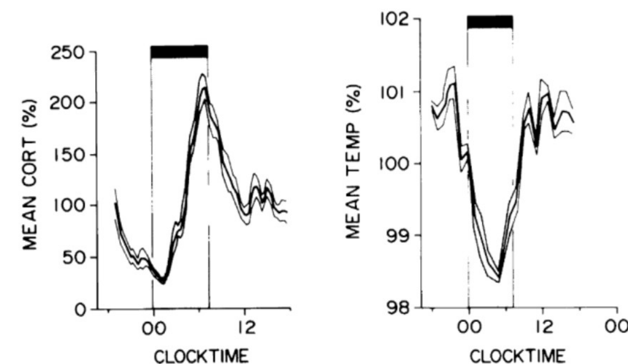

Fig. 6. Mean percentage of cortisol concentration (± SE) and mean percentage of rectal temperature °F (± SE) at 20-minute intervals across basal sleep–wake cycles of normal young men. Data from seven studies of 24-hour duration in six subjects. Details as in Fig. 1.

sleep cortisol and T. Listed in order of exhibition of decreasingly sinusoidal waveform in Figs. 1-7, they rank as T, LH, TSH, PRL, cortisol, and GH.

C. Cosine Fitting and Analysis of Distribution of Resultant Amplitudes and Acrophases

The hormonal time series of each of these 47 studies of ≥24 hours was examined by Halberg's (Halberg *et al.*, 1972) technique of cosine fitting by least squares. The data used for the fits were the first 24 hours of hormonal concentrations at 20-minute intervals that lay between 18:00 and 17:59 the following day. It was reasoned that, rather than calculate the 24-hour period fit to the entire 36- or 48-hour sequence of longer studies, it was more comparably proper to obtain all fits in the same manner and over the same clocktime segment. This would also allow us to test the assumption that our basal state conditions had indeed stabilized possible cues or synchronizers and had minimized stressful transients. The simple test for this would be the extrapolative projection of the fitted first 24-hours' cosine across any remainder of the hormonal times series of raw data. The fit should remain at least as visually good over this remainder as it had been over the initial 24-hour segment, if indeed we had stabilized any existent rhythmicity with a period of 24 hours that lay within the entire hormonal time series.

There were other reasons for the choice of the cosine fitting technique. One is that it does not require equispaced data. Though this was not a problem for our data, it permitted use of unmanipulated raw data in that even small segments of one or two missed samples did not require interpolation. Another reason for its choice was that a time series only 24 hours long is required for fit of the 24-hour cosine. This stands in marked contrast to the powerful techniques of time series analysis which require minimum lengths of just beyond 48 hours to examine the 24-hour periodicity, e.g., autocorrelation (Parker *et al.*, 1979b), variance spectra (Parker *et al.*, 1979b; Halberg and Panofsky, 1961), and average waveform periodographic techniques (Dorrscheidt and Beck, 1975; Parker *et al.*, 1979b). Such techniques would have eliminated about 75% of our time series from consideration. Thus, on purely pragmatic grounds, the choice of cosine fitting technique was easy.

Cosine fitting has some disadvantages for hormonal data. We have already commented on the nonsinusoidal waveform of hormone concentrations in plasma across time. Indeed, the sine (or cosine) seems to be all hormones' least favorite form of expression. Thus, we are assigning a hard task to the method that, through no fault of its own, can only

approximate the original hormonal time series. It is therefore important that an individual cosine fit be compared to its raw data plot for sensible interpretation. However, when used for a group of time series, each of which present relatively the same sort of waveform problem for fitting (e.g., our 26 GH studies), the cosine technique retains its comparative power across the group and yields important, reliable, and replicable information.

The following parameters were obtained from the fit of 24-hour cosines to 24-hour-long segments of our time series of hormonal data of each study.

1. C_0: The midpoint concentration about which the 24-hour long time series oscillates. This usually approximates the linear 24-hour mean hormone concentration.
2. C: The (half) amplitude of the cosine, which is the maximum distance (in units of hormone concentration) above C_0 that the fitted cosine achieves. When expressed as percentage of C_0, it allows comparison between subjects for that hormone and to other hormones. When expressed as percentage $(C + C_0)/C_0$, it centers the fitted cosine oscillation about 100% hormone concentration and becomes analogous to our original percentage of hormone concentration time-series plots. By superimposition this allows visual comparison of raw hormonal and fitted cosine data.
3. Acrophase (ϕ): The temporal locus of the maximum positive amplitude, C, of the fitted cosine. Over 24 hours' 360°, each hour is 15°, and 0° is 00:00 clocktime or midnight.

The percentage C and ϕ results referenced to clocktime for the 24-hour cosine fit to the first 24 hours of hormonal data in each individual study are given in Table III for GH and PRL, in Table IV for TSH and LH, and in Table V for testosterone and cortisol. Nonsignificant individual fits ($p > 0.05$) are marked by an asterisk after the percentage C value in these tables (Halberg *et al.*, 1972).

From the percentage of C and ϕ of each individual study of a hormone come a group of amplitudes and acrophases that represent the distribution of these parameters in all basal state studies of that hormone. The group mean percentage C and ϕ of this distribution can be estimated from the summing of these individual vectors (Halberg *et al.*, 1967). These group mean results are given in Table VI for each hormone. For comparison to these group means of percentage of C and ϕ for each hormone are given the daily maximum of mean percentage hormonal concentration and its temporal locus in angular time of the

TABLE III

Individual Study's 24-Hour Cosine Fits for Growth Hormone and Prolactin Amplitude (C) and Acrophase (ϕ) of Fit to First 24 Hours

		GH				PRL			
Subject	Study	$C_{CT}1$[a]	ϕ_{CT}[a]	ϕ_{SO}[c]	ϕ_{SD}[d]	C_{CT}[a]	ϕ_{CT}[b]	ϕ_{SO}[c]	ϕ_{SD}[d]
1	S_1	64	356	10	87	47	56	70	147
	S_4	25*	10	25	75	28	30	45	95
2	S_1	75	352	2	82	54	70	80	160
	S_4	72	15	14	79	15*	60	59	124
3	S_1	33*	29	33	118	46	18	22	107
4	S_1	57*	332	352	61	36	36	56	125
5	S_1	143	10	18	76	—	—	—	—
6	S_{11}	74	346	2	53	43	42	58	109
	S_7	61	355	7	56	52	37	49	98
7	S_1	128	8	19	110	33	54	65	156
	S_5	67	321	328	22	60	37	44	98
8	S_1	110	331	346	63	16	37	52	129
9	S_2	145	10	24	82	76	83	97	155
	S_1	50*	11	358	76	55	72	59	137
10	S_2	117	345	353	51	36	61	67	127
11	S_3	95	351	9	51	18	46	64	106
12	S_2	107	10	24	75	61	59	73	124
13	S_2	46	6	13	72	31	25	32	91
14	S_3	118	15	24	91	16	43	52	119
15	S_3	19*	271	288	347	30	67	84	143
16	S_3	34*	342	2	59	15	68	88	145
17	S_2	109	1	17	70	8	56	72	125
18	S_2	116	13	15	82	8*	57	59	126
19	S_2	57*	336	349	46	10	32	45	102
20	S_2	76	3	344	73	41	62	43	132
21	S_1	76	337	347	42	36	45	55	110
Vector[e]		76.0	357	7	71	33.1	52	61	126

[a] Amplitude C is given as percentage of 24-hour midpoint (C_0).
[b-d] Reference 0° is midnight clocktime (CT), sleep onset time (SO), or time sundown (SD) for the respective acrophases (ϕ).
[e] Vector—estimate of group mean percentage C and ϕ.
* Nonsignificant cosine fit.

original hormonal time series (e.g., of the "raw" data shown in Figs. 1–6). Also given in Table VI is a precis of the frequency and ranges of distribution of significant fits in individual studies' ϕ for each hormone.

From Table VI, much important information about daily hormonal rhythms is obtained:

TABLE IV

Individual Study's 24-Hour Cosine Fits for TSH and LH: Amplitude and Acrophase[a]

			TSH				LH				
Subject	Study		C_{CT}	ϕ_{CT}	ϕ_{SO}	ϕ_{SD}	Study	ϕ^{C}_{CT}	ϕ_{CT}	ϕ_{SO}	ϕ_{SD}
1	S_5	N_1	55	9	31	84	S_2	9*	329	348	57
		N_2	35	35	48	111					
2	S_4	N_1	25	50	49	114	S_2	23	137	138	225
		N_2	16	25	15	89					
3	S_4	N_1	54	327	329	48	S_2	22	20	30	107
		N_2	39*	357	33	75					
4	S_2						S_2	13	63	68	150
5	S_1		9	265	273	331					
6	S_{11}		3*	75	91	142					
	S_7		22*	284	296	345	S_7	32	53	65	114
	S_3		92	346	343	95					
7	S_1		16	11	22	91					
	S_5		18	40	47	101	S_5	7	30	37	91
8	S_1		31	4	19	96					
9	S_2		29	10	24	82					
	S_1		42	8	355	103	S_1	18	22	9	117
21	S_5		38	354	350	103					
22	S_1		10	356	17	72					
23	S_1		34	17	9	112	S_1	22	60	52	155
24	S_1		24	8	13	68	S_1	31	348	353	48
	S_{20}						S_{20}	16*	26	31	99
25	S_1		4*	355	5	55	S_1	16*	202	212	262
	S_{14}		39	341	336	47					
	S_{20}						S_{20}	4*	9	16	82
26	S_1		5*	357	10	57	S	14	65	78	125
27	S_1		4*	90	95	150	S	15	193	198	253
	S_{20}						S	15*	191	199	264
28	S_{14}		52	342	342	48					
29	S_{14}		30	28	25	94					
30	S_{20}						S	11	346	353	59
	Vector		25.8	359.9	4	82		7.8	42.3	46	122

[a] See footnotes for Table III.

1. 24-hour rhythmicity: The frequency of significant 24-hour fits in these studies is, in the main, higher than would be expected by chance alone: 73% for GH, 92% for PRL, 77% for TSH, 100% for T and cortisol, and 69% for LH. These results indicate 24-hour rhythmicity to be the rule and not exception for all hormones studied under our basal state conditions. The range of distribution of significant acrophases is

TABLE V

Individual Study's 24-Hour Cosine Fits for Testosterone and Cortisol Amplitude (C) and Acrophase (ϕ)[a]

Subject	Study	Testosterone				Cortisol			
		C_{CT}	ϕ_{CT}	ϕ_{SO}	ϕ_{SD}	C_{CT}	ϕ_{CT}	ϕ_{SO}	ϕ_{SD}
6	S_7	29	70	82	131				
7	S_5	25	51	58	112				
9	S_1	40	111	98	206				
23	S_1	14	121	113	216				
24	S_1					54	118	123	178
25	S_1					37	137	147	197
	S_{14}					74	136	131	202
26	S_1					73	149	162	209
27	S_1					49	146	151	206
28	S_{14}					50	147	147	213
29	S_{14}					59	135	132	201
Vector		23.9	88	87	166	55.6	139	142	201

[a] See footnotes for Table III.

also less than 90° and thereby seemingly directional and nonrandom for GH, PRL, T, and cortisol. Such an "eyeball estimate" cannot be made for TSH and LH however. Thus, GH, PRL, T, and cortisol clearly meet our frequency and directionality of significant fit expectations of 24-hour rhythms, while TSH and LH meet frequency but not clearly directionality expectations.

2. Amplitude detectability: Turning from the individual cosine results to the group mean cosine estimates in Table VI, one can estimate that the mean cosine amplitudes are beyond the error of RIA determinations since the mean level of 100% + 2 × (coefficient of intra-assay variation) [GH 9.4%, PRL 7.0%, TSH 4.3% (Azukizawa *et al.*, 1976), LH 2.4% (Yen *et al.*, 1972), T 5.8% (Judd and Yen, 1973), cortisol 2.7%] is still a good deal smaller than mean amplitude (% C) in Table VI. The real maxima in the first column, rather than the fitted cosine amplitudes, show that the mean concentration rise above 100% is larger, most certainly real, and methodologically detectable for all hormones. These concentrations' maxima-cosine amplitude differences also confirm our previous point of cosines only approximately describing hormonal data—as do the differences between the angular clocktime of concentration maxima versus the angular loci of mean acrophases (Table VI).

3. Nychtohemeral 24-hour rhythmicity: The angular clocktimes of

TABLE VI

Basal State Studies' Hormonal Results

	Group study mean				Individual studies		
	Max. 24-hour plasma conc.[a]		24-hour cosine[b]		Signif.	Cosine	Fits[c]
	± SE (%)	Ang. time	C (%)	ϕ	Daily freq.[d]	ϕ range[e]	Noct. freq.[f]
GH	541 ± 57	5°	176	358°[g]	19/26	54°	19/25
PRL	184 ± 18	100°	133	52°[g]	23/25	65°	23/25
TSH	174 ± 10	345°	126	0°[g]	17/22	145°	16/20
LH	121 ± 7	40°	108	42°	11/16	207°	9/12
T	127 ± 7	70°	124	88°	4/4	70°	2/2
Cort.	220 ± 11	105°	156	139°[g]	7/7	31°	0/0

[a] Maximum value of the group's mean percentage concentration for that hormone across the first 24 hours of all that hormone's studies ± standard error, and its clocktime locus in angular time when 0° = 00:00 and each hour = 15°.

[b] Group mean estimate of cosine amplitude C, expressed as percentage $(C + C_0/C_0)$ to make it comparable to the hormonal maximum of the first column; acrophase (ϕ) in degrees for comparison to angular time of the concentration maximum in the second column.

[c] Determined by single cosinor method of Halberg (Halberg *et al.*, 1972) at $p \leq 0.05$ level.

[d] Daily frequency: n of significant cosine fits across the first 24 hour/total n of first 24 hour fits.

[e] Range of circular distribution of these significant acrophases in degrees.

[f] Nocturnal frequency: n of significant nocturnal acrophases/total n of nocturnal acrophases, where nocturnal indicates the ϕ occurred between sundown and sunup.

[g] Determined by group cosinor method of Halberg (Halberg *et al.*, 1967) in which the resultant 95% error ellipse was shown to be significantly different from 0 (intercept).

mean 24-hour hormonal maxima all fell between 345° and 105° (i.e., 23:00 to 07:00). The mean acrophases indicate that all mean hormonal cosine amplitudes occurred between 358° and 88° (i.e., 00:00 to 06:00). The exception was cortisol whose acrophase at 139° locates it at 09:27. The frequency of significant individual cosine fits whose acrophases reside within the interval from sundown to sunrise is 76% for GH, 92% for PRL, 80% for TSH, 75% for LH, 100% for T, and 0% for cortisol. Thus, the mean concentration, mean cosine, and individual cosine frequency results all indicate a common nocturnal locus and close temporal relation to usual (23:00 to 07:00) nightly sleep for all hormones examined except cortisol. The title of 24-hour-long nychtohemeral rhythmicity is clearly established. For cortisol the onset of rise clearly is in noctur-

nal sleep and is preceded by that of its driver, ACTH (Gallagher *et al.*, 1973), so that the labeling of young men's circadian rhythmicity of basal state cortisol as nychtohemerally circadian becomes a matter of semantics.

4. Cosine projection: The group mean cosine for each hormone's first 24-hour pattern has been superimposed upon its mean percentage hormone concentration in Fig. 7, and then projected across the remaining

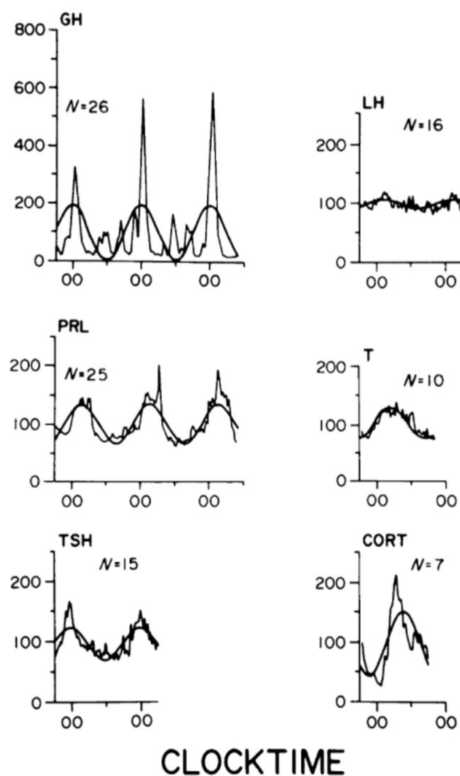

Fig. 7. Group mean hormonal cosine derived from fit to first 24 hours of each study and superimposed on percentage of mean hormone concentration. The hormonal time series across the first 24 hours of each study for that hormone was fitted with a 24 hour cosine. From these amplitudes (%C) and acrophases (θ), the group mean %C, Θ, and cosine were derived. This group mean cosine (thick black smooth curve) of the fit to the first 24 hours was superimposed on the percentage of mean hormonal plot for the first 24 hours (thin black line) in order to visualize goodness of fit. The mean cosine was then projected across the unfitted remainder of this mean percentage concentration plot to test visually continuation of goodness of fit as an index of rhythmicity under our conditions.

unfitted segment of this plot. For GH, PRL, TSH, and LH the projection can be seen to fit the segments beyond the first 24 hours as well as it fits that of the first 24 hours. Our criterion for the successful application of the defined basal state conditions to stabilize any 24-hour rhythms is therein met. There is no reason known to us why T would not behave similarly, and cortisol (Krieger and Aschoff, in press) of course is well recognized to do so.

5. Synchronization: These mean cosine fits to mean concentration plots (Fig. 7) tell us several other important things about these basal state rhythms. One is that the fit horizontally across an entire plot indicates that synchronization of all these hormonal rhythms to a 24-hour period existed *within* those *subjects* whose studies were greater than 24 hours long. The fit vertically of a mean 24-hour cosine derived from the individual fits of a number of subject's first 24-hour studies to the group mean hormonal plot across the first 24 hours indicates that there also is synchronization of these 24-hour nychtohemeral rhythms *between subjects* under our basal state conditions. Each hormone's 24-hour periodicity and synchronization of phase within and between subjects' studies is inferential evidence that these are all basically circadian hormonal rhythms.

6. Statistical significance: Halberg's 95% error ellipse (Halberg *et al.*, 1967) calculated for the study group of cosine parameters for each hormone (Table VI) were significant for GH, PRL, TSH, and cortisol. LH's ellipse just barely overlapped the intercept and T's failed simply because of its small *n*. For example, when our six overnight 12-hour studies of T were fitted to half-24-hour cosines (a technique we do not advocate) and included in the group's T cosine date the T error ellipse achieved significance. Indeed the mean cosine LH and T fits to mean concentration data in Fig. 7 suggest the error ellipse to be a fairly conservative estimate.

A simpler technique is examination of the circular distribution of acrophases that are unweighted for amplitude by the Rayleigh test as redescribed by Batschelet (Batschelet, 1965). The Rayleigh z result is a significance test for directionality or concentration in the distribution of acrophases about their angular mean, since a random distribution of acrophase would be nondirectional and yield low z results. In Table VII, the distribution of acrophases in angular clocktime are seen to be significantly directional for all the hormones. The only nearly borderline result is that for LH. From the Rayleigh test results, an estimate of angular deviation, which is somewhat analogous to linear standard deviation,

TABLE VII

Basal State 24-Hour Hormonal Cosines: Rayleigh Test Results

	Mean ϕ^a	± Ang. dev.[b]	Z^c	p^d	95% Conf. arc[e]
GH	357	±17	22.9	<<0.01	±7
PRL	50	±16	23.1	<<0.01	±7
TSH	6	±36	16.2	<<0.01	±8
LH	36	±60	3.2	<0.05	±51
T	88	±28	3.1	<0.05	±41
Cort.	138	±10	6.8	<<0.01	±10

[a] ϕ, Mean acrophase unweighted for amplitude.
[b] Ang. dev., Angular deviation, the circular equivalent of linear standard deviation.
[c] Z, The test statistic which represents the concentration in distribution of acrophases about their mean. Random distribution yields no concentration and thereby low results.
[d] p, The estimated significance level of Z.
[e] 95% confidence arc, the circular equivalent of the linear 95% confidence interval.

and of the 95% confidence arc, which is somewhat analogous to the linear 95% confidence interval, can be obtained. These are also given in Table VII and are impressively small for all except LH and n-deprived T. Another illuminating result is the relative lack of difference between the mean estimate of the unweighted ϕ here and those of the ϕ weighted for amplitude in Table VI for the same hormone.

Thus, significance tests of group results indicative of 24-hour rhythmicity and directionality (i.e., phase synchronization) are positive for each hormone. Indeed only the LH results were even close to borderline.

VI. PHASE SYNCHRONIZATION OF 24-HOUR-LONG NYCHTOHEMERAL RHYTHMS OF HORMONES: COMPARISON OF CLOCKTIME, SLEEP ONSET TIME, AND SUNDOWN AS BASAL STATE REFERENCE POINTS

The cross-study and between-study synchronization under our basal state conditions was evident in individual study (Tables III–V, VI) and group mean (Figs. 1–7, Tables VI, VII) results. Thus phase synchronization characterized each hormone's results. However, the very uniform nature of our defined conditions mitigate against identification of any

one synchronizing event. For example, our study n is skewed toward summer loci so that we have inadvertently made sundown and sunup time more uniform than would occur in studies distributed more randomly around the year. Another example is that bedtime lights-out and sleep onset on average occurred about one sample interval apart while sleep-offset and arousal from bed also were on average fairly coincident. Thus any change in reference point (away from our clocktime 00:00 = 0° that up until now had been utilized for all analyses) would probably induce only small changes in the group mean plot for that hormone (e.g., maximum's value, locus and variance). However, natural dark onset (sundown, SD) and sleep onset (SO) were precisely known and were more disparate than other available reference points. They were also indices of the most interesting of the likely phasing events, the natural light–dark and sleep–wake cycles.

First the acrophases of the individual fits to the first 24 hours of each hormone were referenced to sleep onset and then to SD. These individual results are found as the ϕ_{SO} and ϕ_{SD} results of Tables III–V. Then the concentration of their distribution about each hormone's mean ϕ was examined by Rayleigh testing (Batschelet, 1965). From this, the mean ϕ, the Rayleigh Z score and the estimate of the length of their mean vector, **r**, were obtained. From these values the 95% confidence arc of mean ϕ could be calculated. A comparative narrowing of the 95% confidence arc would indicate improved concentration in the distribution of ϕ due to use of that reference point, as would an increase in **r.** At 0° dispersion about a mean ϕ, **r** has the length of the radius of a unit circle, 1.0, whereas an **r** of 0.0 indicates a lack of any concentration of ϕ about its mean. The comparative results for clocktime, SO, and SD as reference points in the Rayleigh test are shown in Table VIII. As suspected from the uniformity of our conditions, all differences due to the three reference points were small, and the only z score that failed to achieve significance in directionality was in T referenced to SD. Maximum **r** and minimum arc (which are indicative of the most concentrated distribution of acrophases around a mean) resulted from use of onset of sleep as the reference point for GH, LH, and T. These results fit to some degree with our current notions of sleep-related enhancing effects upon release of these hormones (Parker and Rossman, 1974; Parker *et al.*, 1977). Clocktime proved to be the best reference point under our basal conditions for TSH, cortisol, and, rather surprisingly, for PRL, though here sleep onset ran a rather close second. SD, chosen because it served as a representative of the L:D cycle that is such a major synchronizer in lower mammals and primates, was the worst reference for GH, PRL, LH and

TABLE VIII

Comparison of Rayleigh Test Results When the Acrophases Are Referenced to Clocktime, Sleep Onset, and Sundown for Each Hormone

		Clocktime	Sleep onset	Sundown
GH	ϕ[a]	357°	6°	70°
	Z[b]	22.9	23.2	22.0
	r[c]	0.958	0.963	0.939
	95% arc[d]	±7.2°	±6.7°	±8.7°
PRL	ϕ	50°	60°	124°
	Z	23.1	22.9	22.2
	r	0.962	0.957	0.942
	95% arc	±6.8°	±7.2°	±8.5°
TSH	ϕ	6°	13°	84°
	Z	16.2	15.7	15.9
	r	0.806	0.791	0.797
	95% arc	±16.8°	±17.6°	±17.3°
LH	ϕ	36°	40°	116°
	Z	3.2	3.3	3.0
	r	0.447	0.455	0.436
	95% arc	±50.8°	±49.5°	±52.7°
T	ϕ	88°	88°	167°
	Z	3.1	3.5	2.0
	r	0.877	0.938	0.698
	95% arc	±40.5°	±35.6°	—
Cort.	ϕ	138°	142°	201°
	Z	6.88	6.7	6.8
	r	0.985	0.976	0.983
	95% arc	±9.8°	±12.8°	±10.7°

[a] ϕ, Mean acrophase estimate where 0° = 00:00 clocktime, 0:00 time from sleep onset (first entry into Stage 2), and 0:00 time from sundown, respectively, in each individual study.
[b] Z, Rayleigh Z score (details as in Table VII): all but testosterone's sundown are significant.
[c] **r**, Mean vector estimate as fraction of ideal of one.
[d] 95% arc, the 95% confidence arc of distribution about the mean ϕ.

T, and ran second for cortisol and TSH. These clocktime and SD referencing results suggest that our basal conditions represented a stronger or more uniform set of phasing cues than did SD alone. However, the possible inference that basal phasings of GH, LH, and T are more closely related to sleep onset while those of TSH, cortisol, and perhaps PRL are more relative to the clocktime set of events of our basal conditions should not be carried too far since the differences are relatively small.

VII. SLEEP-RELATED MASKING EFFECTS UPON 24-HOUR-LONG NYCHTOHEMERAL RHYTHMS OF HORMONES

A. Sleep Deletion

Another view of the effect of sleep upon the apparent phase of nychtohemeral hormonal rhythms with a period of 24 hours basally was sought. Here data were gathered across studies in which sleep at the usual time on the first night was not followed by sleep 24 hours later on the second night. The mean percentage hormonal data aligned in clocktime for each hormone are seen in Fig. 8. The individual 24-hour cosine fits to the first 24-hour segments were then used to estimate the group mean percentage amplitude and mean ϕ for each hormone. This group mean hormonal cosine was superimposed upon the first 24 hours of the plot of the percentage of mean hormone data and was projected across the remainder of the data. This was exactly as had been done for basal data in Fig. 7 except that now the "test" projection was across a 6–12 hour remainder that was devoid of sleep. The first thing that is evident in Fig. 8 is that only cortisol behaves as expected of a classic circadian rhythm that would persist seemingly unaltered in phase and amplitude for at least one day after the deletion of a synchronizing signal. Here the only basal state signal deleted was sleep, and if sleep were a classically circadian synchronizing signal its effect should have been upon the (unseen) third night pattern and not immediately upon the second night's hormonal events. In fact, if any of the available basal synchronizing events including sleep were solely and uncomplicatedly critical to phasing, the first and second night's mean % hormonal patterns should have been identical and equally well fit by the mean cosines. That this clearly did not happen, except in the case of cortisol, is evident in Fig. 8.

Since all hormones except cortisol in Fig. 8 do not show evidence of unaltered persistence of basal nychtohemeral rhythmicity in the absence of sleep, these hormones' overt nychtohemeral rhythmicities are either not circadian or are circadian but in a rather complex and obscured form. In favor of the latter would be some evidence of their persistence even in an altered way on the second night, preferably near the clocktime loci of each one's basal acrophase.

Looking first at GH, one simply does not see any convincing sign of the former midnight maximum on the second night. As a result, its mean cosine projection literally has nothing to which to fit, so that GH

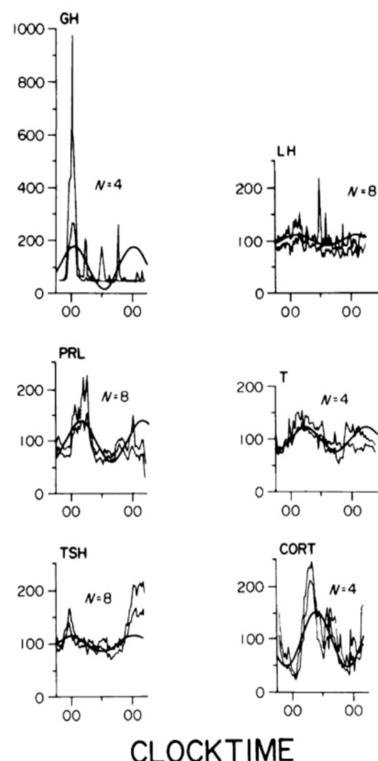

Fig. 8. Immediate effect of missed sleep upon overt phasing of hormonal nychtohemeral rhythmicities. Mean percentage of hormonal concentration (shown as ± SE zone) and group mean cosine of first 24 hours (black curve) were derived as in Figs. 1 and 7, except that for the studies here, the mean cosine of fit to the first 24 hours is then projected across a remainder segment on the second night that is devoid of sleep.

appears noncircadian by this criterion. This is in keeping with results that indicate the nychtohemeral GH maxima only begins after sleep onset and consistently peaks in early sleep (Takahashi *et al.*, 1968; Honda *et al.*, 1969; Parker *et al.*, 1969, 1979b; Parker and Rossman, 1973, 1974). Daily maxima in GH maintain their intrasleep relation in response to delays and advances in sleep onset and immediately shift their phase to maintain their position in sleep regardless of where sleep is placed across the day (Parker and Rossman, 1974; Parker *et al.*, 1979b; Sassin *et al.*, 1969). Thus the daily maximum in GH is clearly sleep-related and is apparently independent of the clocktime of sleep. We interpret this evidence to indicate that the phase of the "circadian" fre-

quency that represents nychtohemeral GH rhythmicity is a dependent variable of sleep, i.e., is dependently entrained to sleep (Parker *et al.*, 1969, 1979b; Parker and Rossman, 1973, 1974). Since sleep-wake cyclicity is an endogenous circadian rhythm in its own right (Weitzman *et al.*, 1975), GH's nychtohemeral rhythmicity appears to be most aptly viewed as a dependent or secondary form of circadian rhythm. The origin of GH's ultradian frequencies is presently unclear (Parker *et al.*, 1979b).

Daily basal maxima in prolactin have also been regarded as sleep-related. The rise, peak, and often the onset of decline are confined to nightly sleep. In addition, daily basal PRL maxima shift to maintain their intrasleep loci in response to advance, delay, or phase reversal of sleep across the day (Parker and Rossman, 1974; Parker *et al.*, 1973; Sassin *et al.*, 1972; 1973). However, it has been claimed, in studies in which sleep has not been objectively assessed, that PRL begins to rise prior to bedtime (Copinschi *et al.*, 1978; Vekemans and Robyn, 1975). In contrast, under our carefully controlled conditions here, even our mean plots across clocktime show a relatively steep ascent to peak in the sleep interval (e.g., Fig. 2 and left of Fig. 8). This rise becomes even more clearly an intra-sleep event if data are aligned by time from sleep onset (Fig. 9) rather than by clocktime. Thus, the daily maximal peak in PRL also strikes us as an almost entirely sleep-related event. However, our earlier results showing a greater tautness of acrophase distribution referenced to clocktime rather than to sleep onset, and, in Fig. 8, the "premissed sleep" segment from nadir to 100% concentration in the mean plot and cosine fit on the second night weakly support a persistent circadian component or a very low amplitude sleep-unrelated component in PRL variation that deserves further consideration.

In the second night's "missed sleep" segment (Fig. 8), mean concentrations of both LH and T deviate from the projected mean cosine shortly after sleep fails to appear. For both hormones there is a preceding nadir—100% segment just before missed sleep that suggests a circadian rise had begun but was then aborted when sleep failed to appear. This is clearer for T than for LH, though of relatively low amplitude for both. We have shown the daily maxima in young adult males' testosterone to shift immediately with reversal of the sleep phase into daytime hours (Parker *et al.*, 1977) but not in the same straightforward fashion as do GH and PRL. This is because residual nighttime peaks in T remain in wakefulness as well. The residual nocturnal-wake peaks in T may represent the circadian component hinted at in Fig. 8. For adult male LH, the existence of sleep-related, nychtohemeral, or circadian components is generally not accepted (Boyar *et al.*, 1972; Krieger *et al.*, 1972; Yen *et al.*, 1974). Only in puberty have sleep-related and sleep-reversible maxima

in LH been seen (Kapen *et al.*, 1974). In this regard it is interesting that with acute sleep reversal, a residual night-wake rise in pubertal LH was also seen (Kapen *et al.*, 1974). Thus, only in puberty is there a clear precedent for the nychtohemeral and sleep-related components in daily LH variation that are suggested to persist in the young men in Fig. 8.

Thus, evidence of altered circadian persistence in the absence of basal sleep is relatively negative for GH, weak but existent for PRL and LH, and moderately impressive for T. However, the evidence for altered circadian persistence in regard to TSH is very convincing. In Fig. 8, the presleep segment of the TSH rise on the first night is faithfully replicated on the second night. Thereafter in the absence of sleep, TSH simply continues to rise to a significantly later and larger maximum. It is the only hormone of the five to have its cosine projection fall below the mean concentration plot rather than vice versa. This continuing rise in TSH in the absence of sleep is converse evidence of the sleep-related inhibition of TSH to which we have called attention (Parker *et al.*, 1976). Finally in Fig. 8, cortisol presents the only evidence of circadian persistence that is unaltered by missed sleep.

The foregoing has summarized, first, the evidence for the circadian character of the 24-hour period in nychtohemeral rhythmicity of these hormones, and second, the evidence that within sleep there exists the capacity to alter immediately hormonal release. From this, we propose that the altered waveforms in the absence of sleep after 23:00 on the second night are due to the immediate absence of such sleep-related effects. It follows that these immediate sleep-state effects upon release may be acting to mask the circadian nature of nychtohemeral hormonal rhythmicity by their stimulatory or inhibitory effects upon the overt manifestation of phase and amplitude of these hormonal rhythms. There is ample precedent for masking effects upon other circadian rhythms (Aschoff, 1979) such that even a single synchronizer may act both to mask immediately and at the same time to synchronize the subsequent phase of the underlying oscillator for a rhythm. Sleep as a masker is an unfortunate likelihood experimentally, since sleep cannot be deleted completely for very long.

B. Sleep Dispersion

We have sought evidence of masking effects in another way however. In Fig. 9 are seen the mean results of pooled 24-hour baseline segments in which sleep onset had occurred at the usual time. The mean results from other 24-hour segments in which sleep onset had occurred across the rest of the day at about 3-hour intervals from 03 to 21 hours are the

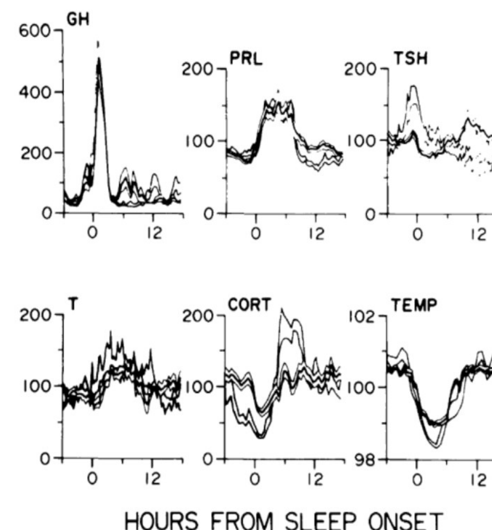

Fig. 9. Masking effects of baseline and nonbaseline sleep upon nychtohemeral hormonal rhythms. Baseline sleep plot (gray ± SE zone): mean percentage of hormonal concentration when aligned for averaging from sleep onset. This sharply focuses on masking effects of sleep in the 0 to +8 hour segment. Since sleep onsets vary little about 23:00 in this group, any clocktime aligned hormonal events persist in the plot. Nonbaseline plot (black line ± white SE zone): similarly derived, but from studies in which sleep onset was dispersed across the day from 03:00–21:00 hours at about 3-hour intervals. Thus the plot represents the average masking effect of sleep (0 to +8 hours) when dispersed across the rest of the day. This alignment from sleep onset for averaging minimizes any clocktime aligned hormonal events.

source of the second or nonbaseline sleep plot for each hormone in Fig. 9. All data for both plots were pooled by time from sleep onset. Because of the relatively narrow range of accepted clocktimes for basal sleep onset (22:00–01:00), this plot contains sleep-onset aligned as well as clocktime aligned hormonal events. The baseline plot from 0 to +8 hours represents basal sleep's masking effect upon basal nychtohemeral rhythmicity.

The nonbaseline sleep plot contains mainly sleep-onset aligned hormonal events, since pooling for averaging from time of sleep onset tends to misalign and cancel out clocktime aligned hormonal events. The nonbaseline plot represents the average masking effect of sleep upon nychtohemeral rhythmicity when sleep has been dispersed across the rest of day, while the plot itself tends to disperse clock-time aligned hormonal events. Thus the average masking effect of nonbaseline sleep is divorced from circadian or clocktime aligned rhythmicity in a visual

but not in any real sense. Similarity of the two plots in the 0- to +8-hour interval after sleep onset indicates a masking effect to be present and to characterize sleep. Direction of the two waveforms in this interval indicates whether the masking effect of sleep is stimulatory or inhibitory. Congruency of the two plots in this interval probably indicates masking to be the sole effect present.

Rectal temperature is shown since it represents a recognized example of the masking effect of sleep upon temperature's circadian rhythmicity (Aschoff, 1979). Here it can be seen that a decline to a nadir in temperature occurs in sleep regardless of where sleep is located around the day.

Congruent baseline and nonbaseline plots are seen in the 0- to 8-hour interval from sleep onset for both GH and PRL. The masking effect is therefore sleep-related, stimulatory and in all likelihood the sole effect present. Sleep-related masking clearly characterizes T rhythmicity as well and is stimulatory. These results are all compatible with our previously discussed results indicative of sleep-related enhancement of release of these hormones. Both TSH and cortisol results in Fig. 9 indicate masking to be sleep-related and inhibitory. The TSH result corresponds to sleep-related inhibition discussed earlier. A similar decline in cortisol in early sleep when the sleep–wake cycle was free running has been reported (Czeisler *et al.*, 1976). Thus, important masking effects of sleep upon hormonal nychtohemeral rhythmicity exist that are stimulatory for GH, PRL, and T and inhibitory for TSH and cortisol.

Discrimination of sleep's masking from phasing effects and identification of synchronizers of these nychtohemeral hormonal rhythms is the next task.

VIII. SUMMARY

Under basal state conditions, nychtohemeral rhythmicity has been demonstrated visually and analytically to characterize GH, PRL, TSH, LH, and T in young adult males, and to be synchronized across time and between subjects under these conditions. Sleep has been shown to have a variable but important influence upon the overt phasing of all these hormones' rhythms and to have masking effects upon their waveforms.

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