ENDOCRINE RHYTHMS ACROSS REVERSAL SLEEP-WAKE CYCLES

Donald C. Parker, L.G. Rossman, A.E. Pekary, J.M. Hershman,
D.F. Kripke, and W. Gibson
San Diego and Wadsworth Veterans Administration Medical Centers
San Diego and Los Angeles, California

That the endogenous sleep-wake cycle has long been the object of man's innate curiosity is reflected in his earliest myths, and that such cyclicity is involved in some way with his well-being seems to be the consensus of this evidence of the collective unconscious. However, it is only in the past decade that we have come to recognize that hormones basic to growth, nurture, development, maturation, reproduction and energy metabolism have patterns of daily variation which are influenced by the human sleep-wake cycle (Parker, Rossman, Kripke, Hershman, Gibson, Davis, Wilson, & Pekary, in press). Since human hormones such as growth hormone (hGH), prolactin (hPRL), luteinizing hormone, testosterone, and thyrotropin (hTSH) drive these physiologically and phylogenetically essential processes, the a priori assumption that such periodic fluxes in these hormonal concentrations subserve these processes is logically inescapable. Indeed, rather impressive evidence that such is the case is seen in the temporal relation of sleep-related rises in the gonadotropin, luteinizing hormone, and as a result, in male hormone, testosterone, to the initiation and evolution of subtended masculine puberty (Judd, Parker, Siler, & Yen, 1974; Parker, Judd, Rossman, & Yen, 1975; Judd, Parker, & Yen, 1977; Boyar, Rosenfeld, Kapen, Finkelstein, Roffwarg, Weitzman, & Hellman, 1974) and the peaking of average daily growth hormone maxima in sleep during puberty (Parker & Rossman, 1973; Parker & Rossman, 1974; Parker, Rossman, Kripke, Gibson, & Wilson, 1979) to the subtended rapid increase in stature that occurs at that time. These latter strike us as significant physiologic and endocrinologic "performance." There is little question that hormones have important and often rather direct effects upon behavior. However, for clarity, simplicity, brevity and limitation of interpretative scope at this time, here we ask you to forego your own preferred views of performance and behavior and allow flux in plasma concentrations of these potent hormones across the day to represent the observed daily performance or behavior itself. Restated, the presumption that daily endogenous fluxes in hormonal concentration have some influence upon how that hormone exerts its effect(s) upon subsequent behavior does not strike us as an unreasonable thesis that requires an unwarranted leap of faith. After all, relation of externally applied stimuli to subsequent complex and externally observed behaviors requires a similar but much larger leap of faith in cause-effect assignment.

Here we will show the effects of phase reversal of the sleep-wake cycle upon the endogenous daily patterns of release of man's growth hormone, prolactin, thyrotropin, and cortisol. This latter is a reflection of its pituitary tropin, ACTH (Gallagher, Yoshida, Roffwarg, Fukushima, Weitzman, & Hellman, 1973; Krieger, Allen, Rizzo, & Krieger, 1971).

Basal State Conditions

Our observations were made in 8 healthy young adult males between the ages of 19 and 28. Our sampling technique consists of the drawing of 2 ml volumes of blood at 30 minute intervals from an indwelling antecutibal venous catheter

across 24-72 hour sampling periods. All hormones are measured by sensitive, specific and stable radioimmunoassays in the same plasma sample (Parker et al., in press; Pekary, Hershman, & Parlow, 1975). Such intracaths were painlessly emplaced, carefully secured and dressed, and maintained patent by a heparin-lock system. A small bore 10 foot extension line (void volume 1.8 ml) allowed sampling from outside the bedroom. Sleep was polygraphically recorded and scored by standardized techniques (Rechtschaffen & Kales, 1968). The room was not entered nor the subject's sleep disturbed by intrasleep sampling. Wakefulness was carefully supervised by us and consisted of sedentary wakeful activity, regular equispaced feedings and minimization insofar as possible of intercurrent stresses. Subjects were not permitted to lie in bed or nap during wakeful segments and were exposed to usual social and environmental cues during wakefulness. Awareness of cues to 24 hour clock time was maintained by radio and TV programming, clocks, watches and visiting schedules. Basal sleep schedules ran from 2300 to 0700 hours and were maintained by all subjects at home prior to study, during an accommodation day and during subsequent days under these basal state conditions. Six of 8 subjects also had additional 24-36 hour long segments of basal state data available.

Sleep-Wake Reversal

Reversal of the sleep phase was achieved by delaying bedtime from 2300 hours until 1100 hours on the first day of reversal and then holding the bedtime sleep segment fixed in this 1100-1900 hour interval thereafter. In all wakeful segments across both basal and reversal studies, all subjects were exposed to the available natural light phase of the LD (Light Dark) cycle. On all basal and reversal study days the artificial illuminative schedule conformed to the respective wake phase. Four subjects were studied in an accute SW (Sleep Wake) reversal protocol which consisted of a 72 hour sampling period of serial 24 hour long (1900-1900) basal (B), first (R_1) and second (R_2) reversal segments. They were fed the same meal three times per day at 0730, 1230 and 1730 basally. This 10 hour feeding segment was phase-reversed to 1930, 0030 and 0730 during sleep-wake reversal. This group slept in full darkness in a bedroom from which natural light was excluded during the 1100-1900 sleep segment in the R_{1-2} reversal period. The natural LD cycle was 14:10 (2 subjects: July; sunset 1956 PST) and 11:13 (2 subjects: February; sunset 1738 PST).

The other 4 subjects who continued their sleep-wake reversal schedule for 15 days were sampled in a basal 24 hour segment from 1900 to 1900 and then during three 60 hour long periods across the sleep-wake reversal: "R_{3-5}" was from 1900 of R_2 to 1100 hours of R_5; "R_{8-10}" was from 1900 hour of R_7 until 1100 hours of R_{10}; "R_{13-15}" was from 1900 hours of R_{12} until 1100 hours of R_{15}. The "long" reversal's subjects were fed the same meal every 2 hours beginning at 0800 until 1800 basally so that the 24 hour ration of calories was taken as 6 equal feedings in wakefulness. During sleep-wake reversal, this 10 hour long feeding segment was phase reversed to run from 2000 until 0600. During the 15 day long reversal period, natural light was <u>not</u> excluded from the sleeping quarters during their 1100-1900 bedtime sleep segment. This study was carried out in September when the LD cycle was 12:12 and sunset at 1900 PST. Thus, the differences between the acute and long reversal studies were in subjects, feeding schedule and the availability of natural light in daytime sleep segments during the sleep reversal periods.

Data Calculation and Display

a) Raw hormonal data: The mean hormonal concentration for each 24 hour Basal, 48 hour R_{1-2} or 60 hour long R_{3-5}, R_{8-10} and R_{13-15} segment for each hormone in each subject was calculated and every one of his hormonal concentrations at 30 minute intervals expressed as a percent of this mean. This represents an attempt to "normalize" variation between subjects. Then the subjects' percent concentration data were pooled at 30 minute clocktime intervals across that segment for that hormone to arrive at the group's mean percent concentration plot across clocktime of that basal or reversal segment. This permits hormonal events that are synchronized both across 24 hour clocktime and between subjects to stand out. These are shown as Figure 1, 5, 7 and 11 for GH, PRL, cortisol and TSH, respectively, in an actigraphic representation. An individual's cortisol and TSH results in this format are seen as Figure 9, 10, 13 and 14, respectively.

b) Cosine fits were done according to the technique of Halberg (Halberg, Johnson, Nelson, Runge, & Sothern, 1972) to full 24 hour (1900-1900) segments of the data such as Base, R_1, R_2, R_3, R_4, etc., but not to segments with less than 24 hours of data such as R_5 (16 hours from 1900 to 1100 or R_5). The acrophase results are shown in Figure 3 for the individuals' daily acrophases across the reversal.

c) Rayleigh testing for evidence of significant directionality of such groups of acrophases (B, R_{1-2}, R_{3-4}, R_{8-9}, R_{13-14}) as developed by Batschelet (1965) were also done, and the circular mean ō and 95% confidence arcs of the resultant mean acrophases also estimated for each segment of the studies. These appear as Figure 4.

d) The autocorrelation function (Parker et al., in press; Parker et al., 1979) of each 48-60 hour long reversal segment (R_{1-2}, R_{3-5}, R_{8-10}, R_{13-15}) was calculated for each subject at 30 minute lags and the group's mean plot of the autocorrelation function then obtained at each such point across the reversal for each hormone.

e) The variance spectra (Parker et al., in press; Parker et al., 1979) of each individual's reversal segments were also calculated at 1 c/d resolution and from these, the group's mean variance spectra calculated across R_{1-2}, R_{3-4}, R_{8-9} and R_{13-14} segments of the reversal for each hormone (Parker et al., 1979). The mean autocorrelation function and variance spectra results for each hormone are shown in Figure 2, 6, 8 and 12. Since the specific basal segments of the present acute and long reversal studies are too short to permit the autocorrelation function to reach events 24 hours apart they are not shown. We have published normative data from other subjects studied under identical basal conditions (Parker et al., in press).

Growth Hormone

Somatotropin or hGH was the first hormone to be shown to have a relationship between its daily 24 hour maxima and daily sleep (Quabbe, Schilling, & Helge, 1966; Parker, Mace, Gotlin, & Rossman, 1968; Takahashi, Kipnis, & Daughaday, 1968; Honda, Takahashi, Takahashi, Azumi, Irie, Sakuma, Tsushima, & Shizume, 1969; Parker, Sassin, Mace, Gotlin, & Rossman, 1969; Sassin, Parker,

Mace, Gotlin, Johnson, & Rossman, 1969). In Figure 1's actigraphic format can be seen this relationship over the most prolonged period of sleep-wake reversal yet shown. This group mean plot faithfully represents each individual's plots. The basal daily peak early in nocturnal sleep is seen to shift immediately and completely into early daytime sleep on R_1 and then to hold in this position over the rest of the 15 days of sleep-wake reversal. Scanning down the midnight line in the early segments of sleep-wake reversal (R_{1-5}) offers little evidence of a residual peak that might have been circadianally synchronized. However both basal and reversal hGH patterns show multiple peaks in wakefulness whether fed 3 meals/day (R_{1-2}) or every 2 hours (R_{3-15}) in wakefulness. However, the second major synchronized peak of the day during reversal also tends to occur in late sleep. This will later be seen to influence the acrophasal loci of fitted 24 hour-long cosines, as does the nonsinusoidal waveform (principally the duration) of the early sleep maxima.

This immediate reversal of daily hGH maxima with reversal of the sleep phase demonstrates what endocrinologists have been calling sleep-related, sleep-enhanced, sleep-augmented, sleep-stimulated, sleep-dependent or sleep-entrained hGH release. This has been rather loosely referred to as the "circadian rhythm" in hGH variation though we have used the term nychtohemeral (night>day) rhythmicity for basal state data. Perhaps most appropriate of all nominatives is "sleep-wake rhythmicity of hGH." In rhythmic terminology, this hGH rhythm may be seen as that resulting from a rather pure masking effect (Aschoff, 1979; Aschoff, 1960; Aschoff, Hoffman, Pohl, & Wever, 1975) of sleep that is stimulatory.

In Figure 2 are seen the group mean autocorrelation functions and variance spectra for hGH across the reversal segments. The principal difference in the autocorrelation function across the reversal is not that of the constant persistence of 48 lag (e.g., 24 hour) maxima (which also exists in basal state means) but in the midrange of the functions of R_{1-2} compared to R_{3-15}. We suspect this to be due to the difference in feeding schedules of the acute and long reversal protocols such that a masking effect of feeding that is inhibitory has been increased in frequency between the 2 sleep-wake reversal conditions. A persistent circadian component should have shown itself as a 24 lag peak in the reversal's autocorrelation functions. Mean basal variance spectra peak at 2-3 c/d. Across the reversal, the spectra are advanced slightly toward faster frequencies initially (R_{1-4}) and then resume their basal configuration (R_{8-15}). These faster frequencies are based both in the need of the Fourier technique to fit the waveform of the sleep peak and in the real releasing activity in wakefulness and the real appearance of second hGH peaks in sleep during reversal. Thus, despite the easily visualized 24 hour periodicity in raw GH data, the variance spectra correctly attributes larger components of the variance to frequencies > 1 c/d in both basal and reversal states. Thus GH's variation is multifrequential in both conditions.

In Figure 3 is seen the actigraphic display of daily hGH acrophases (ϕ) of 24 hour-long cosines fitted to 24 hour-long (1900-1900) segments across the course of reversal. The immediate delay and then fixation of the acrophasal loci for hGH is readily seen, as in the tautness of their distribution at each point in the reversal. In Figure 4 are plotted the group daily mean ϕ and their 95% confidence arcs for each segment across the reversal. Here it can be seen that the mean R_{1-2} acrophase shift is a delay and is significantly

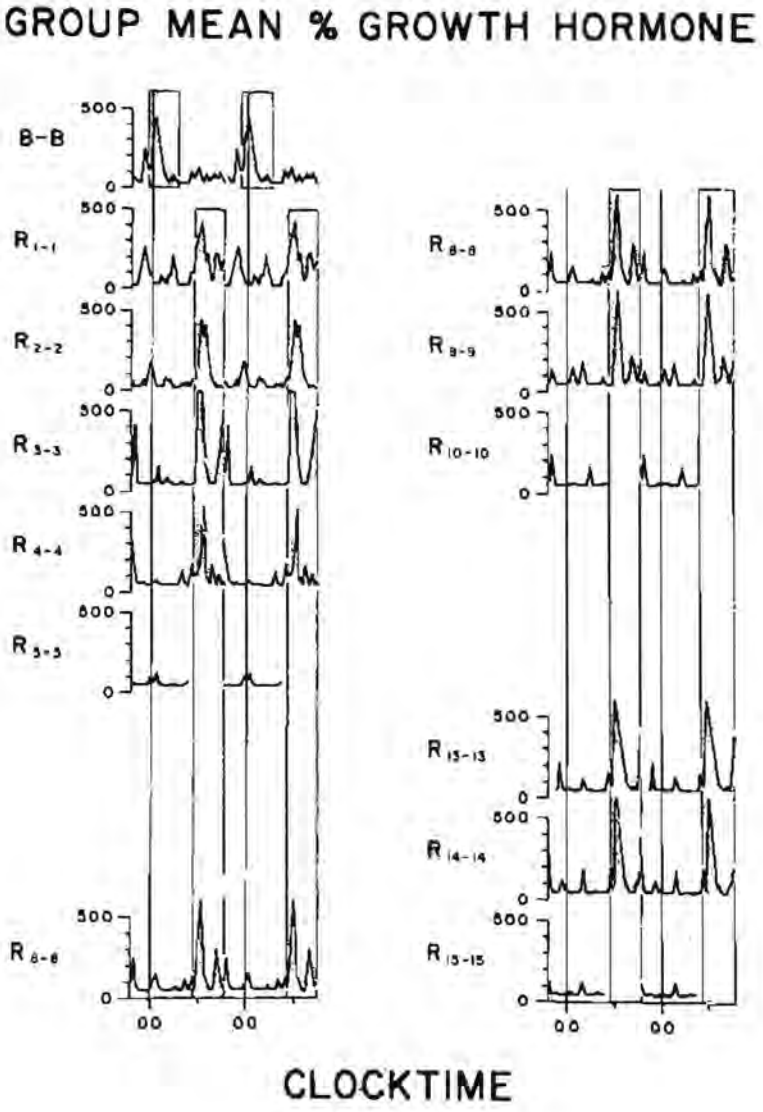

Figure 1. Actigraph of group mean % hGH concentrations across 15 days of sleep-wake reversal. Each hormonal data point at 30 minute intervals was first expressed as % of that subject's 24 (basal, B), 48 (R_{1-2}) or 60 hour (R_{3-5}, R_{8-10}, R_{13-15}) mean for that hormone, and the resultant % of sequential mean data was then pooled for daily averaging at 30 minute intervals from 1900 to 1859. The $\pm$SE bars have been omitted. Eight subjects furnished 14 basal days of data and 4 subject's daily patterns were represented in every daily reversal mean. The gray bars on 8 hour bedtime sleep segments (23-07 basally and 11-19 in reversal) and the vertical black lines are midnight (0000) lines in the doubly plotted (B-B, R_{1-1}, etc.) format. this allows events synchronized across time and between subjects to stand out and their movement across 24 hour time seen.

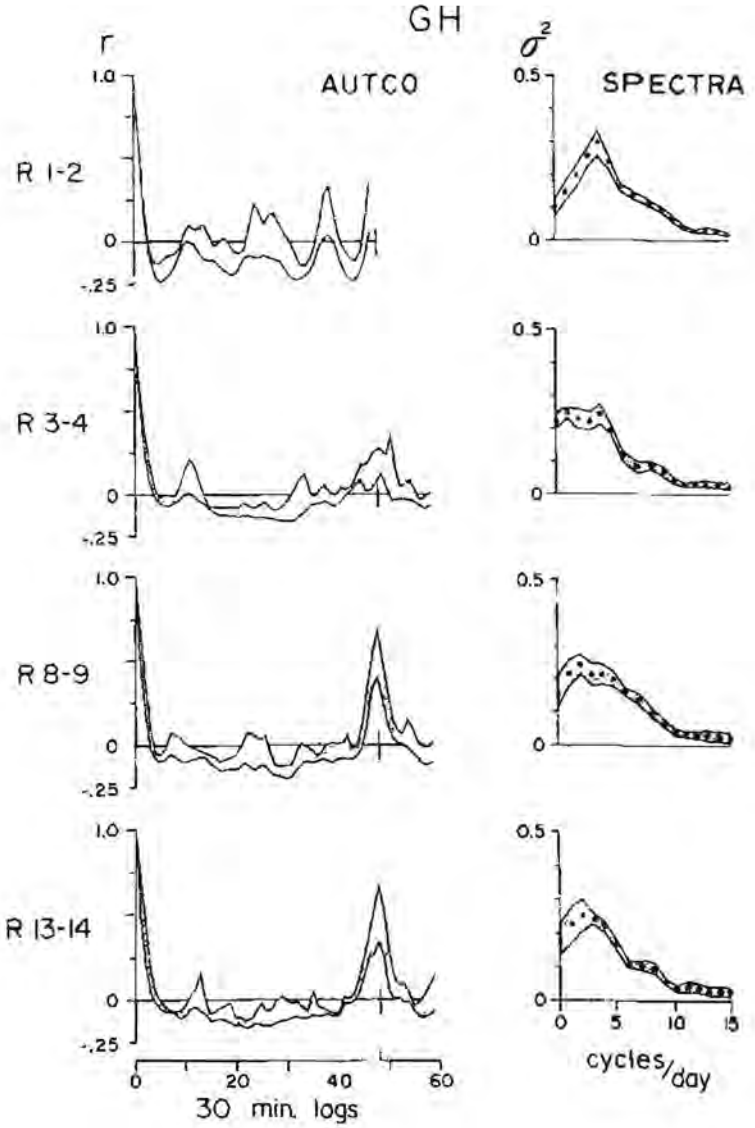

Figure 2. hGH: Group mean autocorrelation (Autco) functions and variance spectra across sleep-wake reversal segments. Autocorrelation functions at 30 minute lags were collected across the 48 hour (R_{1-2} and the 60 hour $R_{3\ 5}$, R_{8-10} and R_{13-15}) segments for each hormone in each individual. Then the group's averages were calculated at each lag for each reversal segment. The gray zip line plot shown is the mean ±SE zone. The variance spectra at 1 c/d resolution were similarly correlated, averaged and plotted.

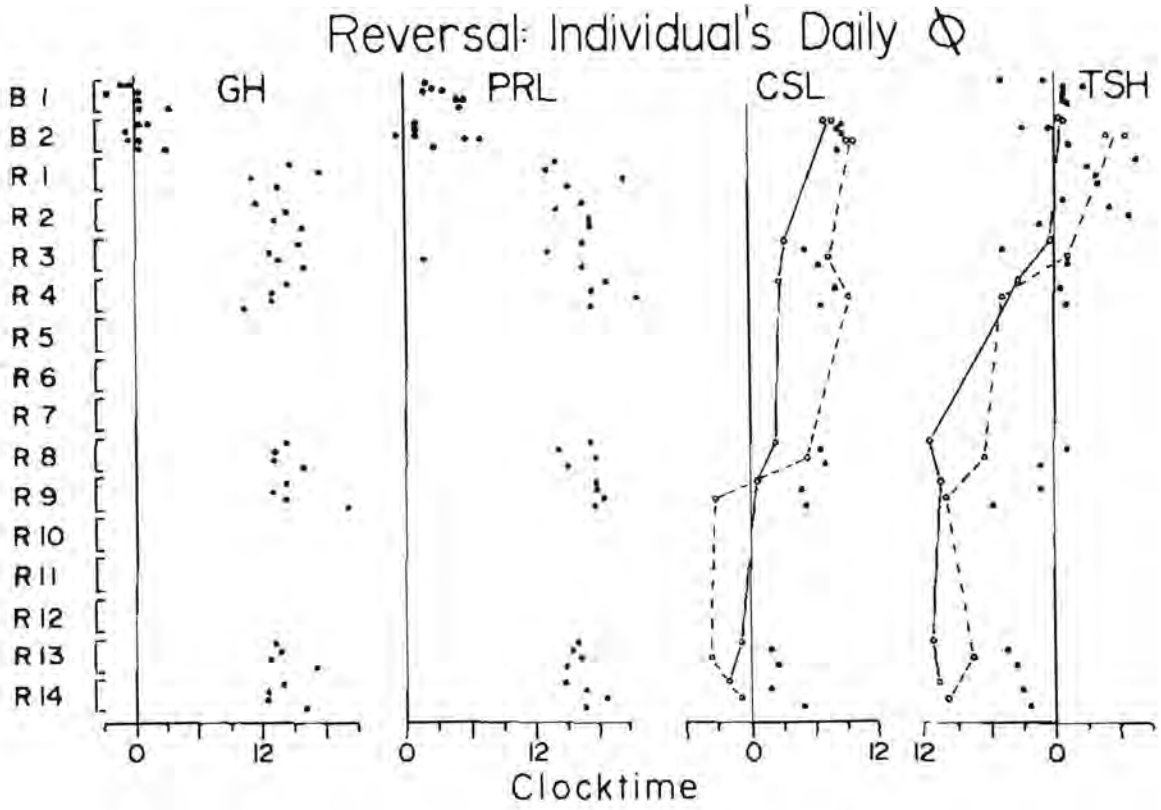

Figure 3. Eight individuals' daily 24 hour cosinor acrophases across sleep-wake reversal: Growth hormone, prolactin, cortisol and thyrotropin. Results have been converted into clock-time from 1900 to 1900 hours of the fitted segment. Basal sleep = 23-07, reversal sleep = 11-19. The solid and dotted lines connecting the open circles represent the phase shifts of subject 300 and 315, respectively.

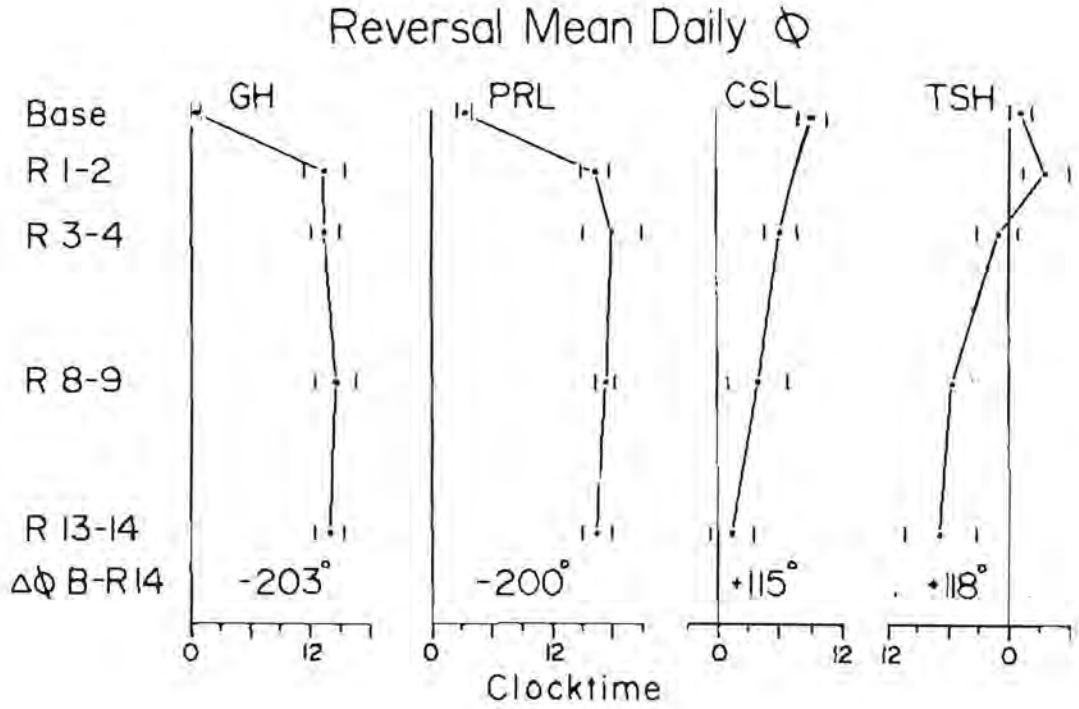

Figure 4. Groups mean daily acrophases 95% confidence arc for hGH, PRL, cortisol and TSH across sleep-wake reversal segments. Cosinor o's from Figure 3 had their circular mean, 95% confidence arc calculated for Basal, R_{1-2}, R_{3-4}, R_{8-9} and R_{13-14} segments. Rayleigh testing for significant directionality of each mean was also done. The $\Delta\phi$ B-R_{14} is the difference between the basal and R_{13-14} means.

different from the basal mean, while subsequent mean R_{3-4}, R_{8-9} and R_{13-14} acrophases are significantly similar to R_{1-2}. We interpret the phase-shift beyond 180° in the ultimate phase delay from basal condition here to be attributable to the displacement of acrophase away from reversals' raw data display of early sleep maxima in hGH that was induced by the addition of second major episodes of hGH release later in daytime sleep during reversal.

Prolactin

PRL, the human lactational hormone, was the second hormone shown to have a relation of daily maxima to sleep (Parker et al., in press; Sassin, Frantz, Weitzman, & Kapen, 1972; Parker, Rossman, & Vanderlaan, 1973; Sassin, Frantz, Kapen, & Weitzman, 1973). The onsets and offsets of maximal release appear to us to be more closely related to sleep itself under basal state conditions than to clocktime itself. However, some who have not objectively monitored sleep have suggested that the onset and offset of the nocturnal peak in PRL extends beyond the confines of basal bedtimes and interpreted this as indicative of sleep-unrelated circadianess in its variation (Vekemans & Robyn, 1975; Nokin, Vekemans, L'Hermite, & Robyn, 1972; Copinschi, DeLaet, Brian, Leclercq, L'Hermite, Robyn, Virasoro, & Van Cauter, 1978). Therefore, examination of prolactin's response to phase reversal of the sleep-wake cycle is of interest in clarifying the determinants of its daily pattern of variation.

Seen in actigraphic format in Figure 5 is the group mean percent PRL data across the reversal, which as for GH, faithfully represents individuals' data plots and shows events synchronized across time and between subjects. Daily maxima in relation to sleep consistently characterize both basal and reversal conditions, where the onset of rise and segments > 100% are clearly restricted to sleep, and declines follow the end of the sleep interval regularly. This all reinforces our point of the strength of the sleep-enhanced (or masking effect of sleep that is stimulatory) contribution to PRL's patterns of daily variations in young men. The immediate shift of the PRL maxima with the sleep phase on R_1 and its subsequent fixation thereafter to the sleep phase across the reversal both resemble the pattern of shift that hGH had undergone. Scanning down the 19-00 interval early in the reversal for evidence of a residual peak in PRL release that could represent an unmasked (from sleep) and synchronized circadian component, one infrequently encounters release that rises above the 100% line (R_{4-5}) nor in individuals' plots did one see much temporal synchrony here. Throughout the latter half of reversal one sees even less evidence of a nocturnal peak in wake that has remained synchronized by the natural LD to which exposure had been maintained. Thus if a non sleep-related circadian component to PRL variation exists, it must be of low amplitude and thereby relatively easily obscured.

The group mean autocorrelation functions and variance spectra across the reversal are seen in Figure 6. The autocorrelation maintains the same 48 hour lag maxima (e.g., 24 hours apart) that characterizes basal state across the entire reversal. The only appreciable change is the appearance of a lessened nadir in negative autocorrelation in the mid-range of R_{3-4} and R_{8-9}. This latter causes the shift in peak of the variance spectra away from the 1 c/d peak of B, R_{1-2} and R_{13-14} to 2 c/d in the R_{3-4} and R_{8-9} spectra. One surmises from Figure 5 that the suggestion of bimodality in the PRL patterns of R_4 and R_8 and their lessened amplitudes in sleep are the source of this minor change

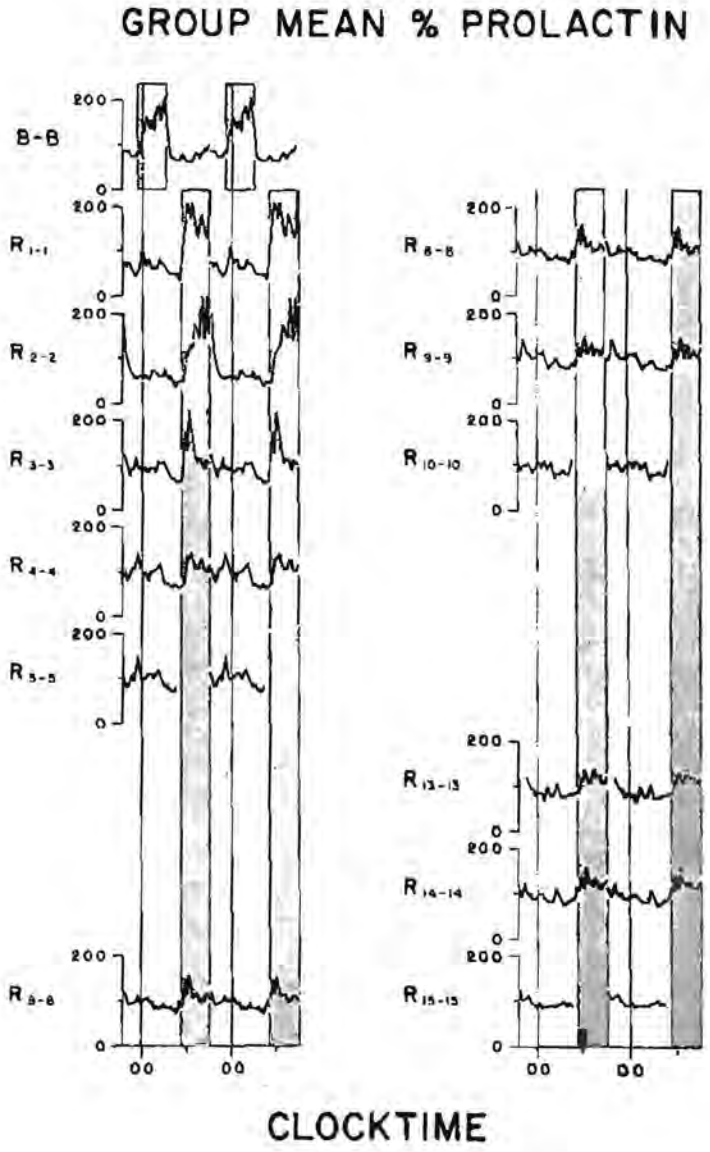

Figure 5. Actigraph of group mean % PRL concentrations across 15 days of sleep-wake reversal. Details as per Figure 1.

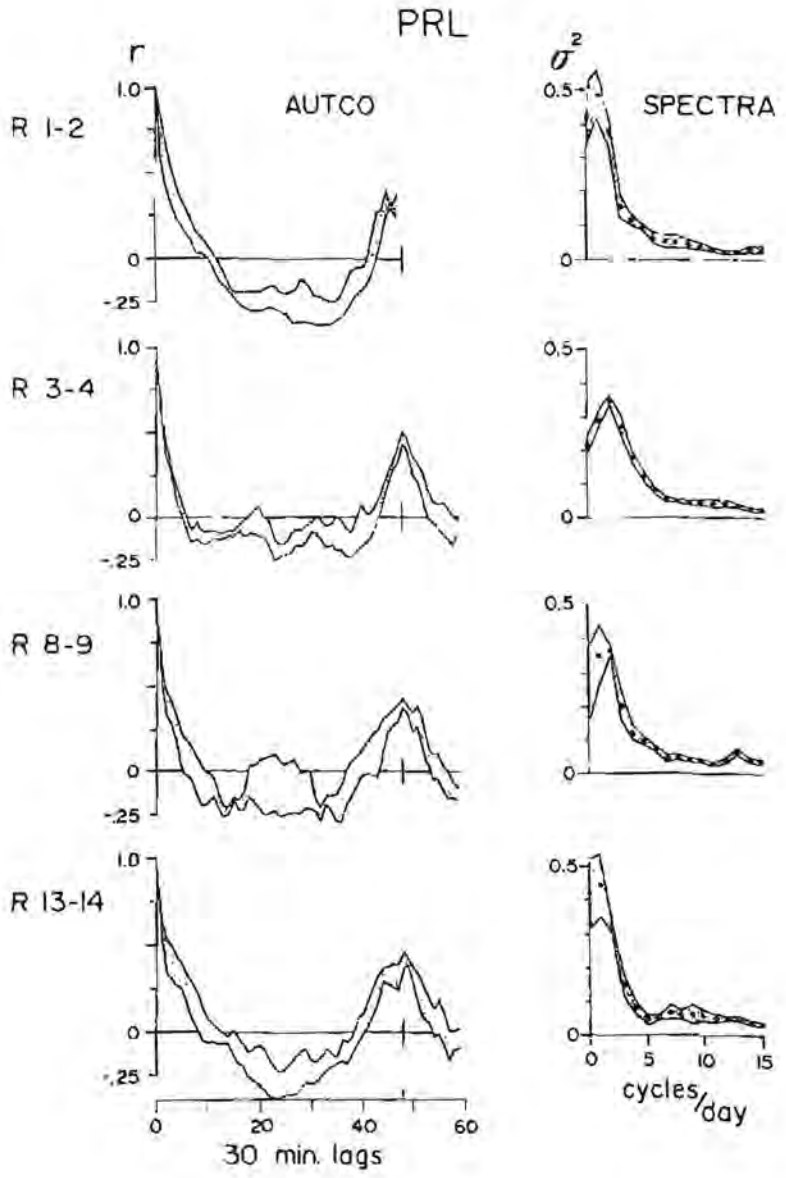

Figure 6. PRL: Group mean autocorrelation (Autco) functions and variance spectra across sleep-wake reversal segments. Details as per Figure 2.

in the autocorrelation and variance spectra. However this may be seen as weak evidence of a residual circadian component that exists in addition to the sleep-related component in the 24 hour PRL variation patterns. Again, one would like to see them persist across the entire reversal or to gradually be lost from R_{1-2} onward to be convincing evidence of circadianess.

The acrophases of the individuals' 24 hour cosine fits to PRL are seen in Figure 3 and are akin to those for hGH. An immediate delay and then fixation across the reversal as well as a nice tautness of distribution are evident. Thus the cosine fits cannot identify any circadian persistence of acrophases in the 23-0700 interval during reversal.

The group mean acrophases for PRL and their 95% confidence arcs across the reversal are seen in Figure 4. All exhibited significant directionality in Rayleigh testing as had those for hGH. The mean ϕ for all reversal segments were significantly different from the mean basal ϕ but were without significant differences within the reversal segments. The confidence arc of the ultimate shift achieved by R_{13-14} of -200° encompassed an 180° delay from the mean PRL ϕ of basal sleep-wake state.

Thus it seems to us that PRL variation exhibits a 24 hour rhythm attributable to a masking effect of sleep itself that is stimulatory and that the evidence of any additional circadian component is somewhat weak. If the latter exists, it is of a low amplitude and easily obscured.

Cortisol

The daily pattern of plasma cortisol variation was the first recognized hormonal circadian rhythm (Tyler, Migeon, Florentin, & Samuels, 1954; Migeon, Tyler, Mahoney, Angel, Castle, Bliss, & Samuels, 1956) and remains today the classical endocrinolgic prototype of such rhythms. Though the light-dark (LD) cycle appears well established as the principal environmental synchronizing cue in the laboratory animal, the demonstration of the importance of the LD cycle in maintaining synchrony of cortisol's endogenous circadian oscillator in man is unclear (Krieger, 1979). For example, blinded humans usually exhibit 24 hour rhythm rather than a free-running one (Weitzman, Perlow, Sassin, Fukushima, Buralk, & Hellman, 1972); when sighted humans are kept in constant darkness but exposed to 24 hour-periodic social cues, again periodicity and phase are maintained (Aschoff, Fatronska, Gisdke, Doerr, Stamm, & Wisser, 1971 1971). However, man usually conforms his sleep-wake cycle to the available LD cycle. Thus transmeridian flights include a shift of all available environmental and social cues as well as that of the elected sleep-wake cycle. Weitzman, Goldmacher, Kripke, MacGregor, Kream, and Hellman (1968) have shown that after laboratory reversal of the sleep-wake cycle for between 1-3 weeks, the phase of the plasma cortisol rhythm becomes reversed. There was considerable disruption of the 24 hour cortisol patterns during the sleep-wake reversal period until its phase-reversal was achieved. Krieger has shown that reversal of the sleep-wake cycle in constant light results in phase reversal of the cortisol rhythm (Krieger et al., 1969). Thus it is clear that there are important sleep-wake effects upon the phasing of man's endogenous cortisol rhythm. In men living on a 1 hour: 2 hour sleep:wake and dark:light schedule, Weitzman, Nogeire, Perlow, Fukushima, Sassin, MacGregor, Gallagher, and Hellman (1974) have seen not only a 3 hour periodicity imposed upon cortisol

but also saw a persistent 24 hour circadian pattern to underlie this. From the foregoing, we suspect that both the sleep-wake cycle and environmental synchronizers play some role in the phasing of the cortisol rhythm. Because of this and because we were curious to know what was happening to the cortisol patterns before full phase reversal was finally achieved, we elected to examine cortisol patterns over the course of our 15 day reversal study.

In Figure 7 are shown the group mean plots of daily 24 hour cortisol patterns across the reversal in an actigraphic format. The average basal pattern is seen to be basically an unimodal nonsinusoidal waveform. Glancing down the daily mean actigraph one sees lesser amplitude bimodal release on R_{3-5}, less clearly organized or synchronized patterns that are bimodal (or more) on R_{8-10} and resumption of a basically unimodal pattern on R_{13-15} whose waveform is still different from basal in that it is basically sinusoidal but slightly bifid. If one follows the basal circadian peak near 0700 down the actigraph one sees it to largely hold its position until about R_{10}, whereafter it appears to slightly advance. Then running the eye down the 1700-00 interval (e.g., late-sleep and post-sleep segments during reversal) one sees increasing amplitude and duration of mean cortisol release here and finally a melding of this with the previously described "basal circadian" peak that has advanced. This suggests that more than just advance (or delay) of a single circadian oscillator for cortisol may be involved in the ultimate achievement of the expected phase-reversal.

In Figure 8, the midranges of the group mean autocorrelation functions for cortisol across the reversal segments also indicate the presence of bimodal components on R_{3-5} and R_{8-10} that have largely "dropped out" by the R_{13-15} remelding. The 48 lag maxima indicates persistence of a predominant 24 hour correlation across all the reversal segments. Peaks in the variance spectra also shift to 2 c/d on R_{3-4} and R_{8-9} before resuming the basal 1 c/d peak on R_{13-14}. This synchronizing of bimodal peaks in Figure 7 and of midrange peaks in autocorrelation in Figure 8 caution against regarding these trends as simply those of noisy transients during reversal.

Turning to the group mean results of cosine fitting, in Figure 4 we see that significant directionality by Rayleigh testing is achieved by each group of acrophases (B, R_{3-4}, R_{8-9}, R_{13-14}) and that a significant advance away from the basal distribution is achieved by R_{8-9}, despite the latter's having the broadest 95% confidence range and therefore probably the greatest disparity in patterns of shift. The group's mean acrophases show a slow gradual advance across the reversal to achieve about an 8 hour advance by the 14th day of sleep-wake reversal. However, since we have shown you hormonal and autocorrelative patterns that are bimodal, one needs to be cautious in interpreting 24 hour cosine fits since the cosine is a test of unimodal fit. Bimodal peaks close together are fit relatively well by a cosine, whereas those 12 hours apart are not as well described. However, this did not prove to be a problem with individuals' 24 hour cosine fits to cortisol as only one of 31 basal and reversal days (315: R_8) was not significantly fitted. A glance at the mean cortisol concentration actigraphic in Figure 7 indicates that the group mean acrophases of Figure 4 are in the main following cortisol's "basal circadian peak" component as it advances.

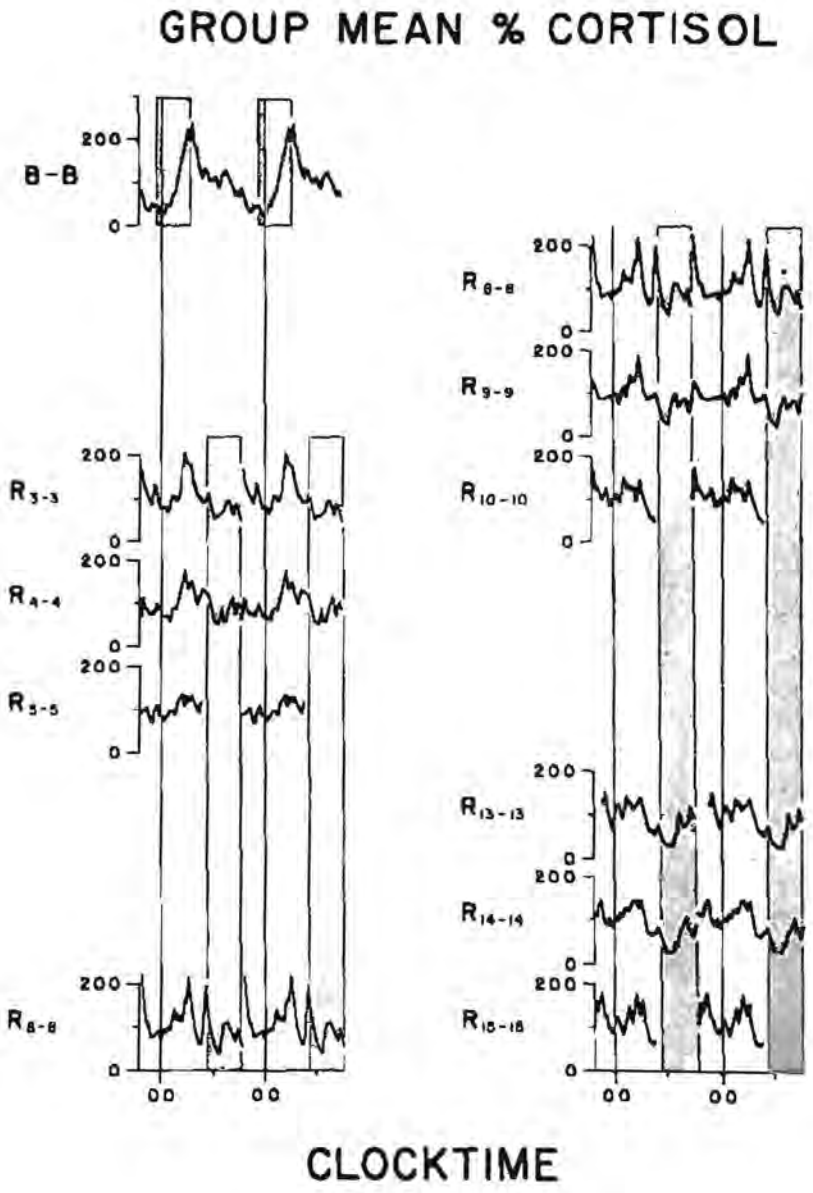

Figure 7. Actigraph of group mean % cortisol concentrations across 15 days of sleep-wake reversal. Details are as per Figure 1 except that the basal groups' plot is from 7 days of 4 subjects who have had no R_{1-2} cortisol data.

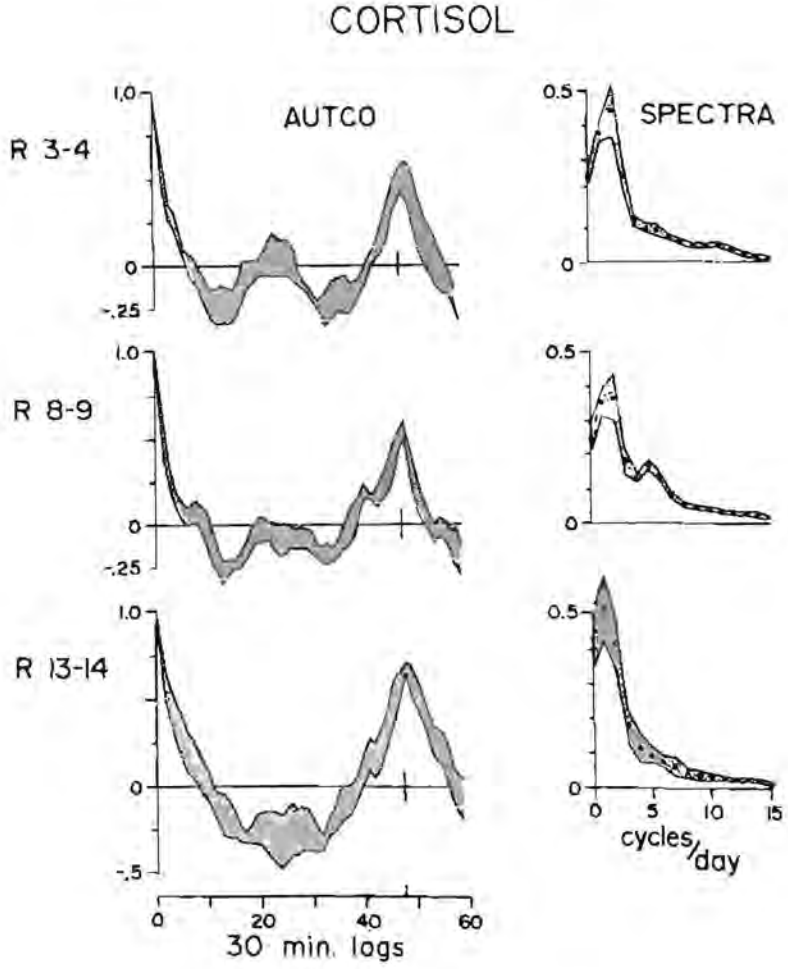

Figure 8. Cortisol: Group mean autocorrelation (Autco) functions and variance spectra across sleep-wake reversal segments. Details as per Figure 2.

The individuals' daily acrophases across the course of reversal (Figure 3) reveal that the pattern of shift in cortisol maxima occurred in at least 3 ways; (1) two (305, 311 - black dots on Figure 3) largely held their basal circadian acrophase position through R_{8-9} and then advanced only minimally by R_{13-14} so that a total advance of about only 90° was achieved. The cortisol actigraph for one of these subjects (305) is shown in Figure 9. Here a bimodal basal pattern (which was also seen in his autocorrelation functions) is largely maintained across the reversal so that the larger "basal circadian" component has advanced its peak only 4-5 hours while the second lesser basal afternoon-wake component was delayed initially and then persisted as the late- and post-sleep component without ever gaining in amplitude or duration and not melding with advancing basal circadian component by R_{15}. Thus a fully reversed pattern was not achieved by R_{15}. The second subject (311) largely held a unimodal pattern throughout (also seen in his autocorrelation function) in which his major basal circadian component held with only slight advances while he failed to organize a consistent late- and post-sleep component. Thus these 2 had not reversed their patterns and had advanced only slightly by Day 15.

Of the 2 who reversed their patterns more fully, each exhibited a different pattern. (2) one, 300, shown actigraphically in Figure 10, exhibited basically a unimodal pattern (also seen in his autocorrelation functions) across the reversal in which the basal circadian maximum steadily advanced to complete the phase shift by R_{13-14}. His acrophases are the open circles connected by a solid line on Figure 3 and accurately replicate these cortisol concentration data results. (3) the other subject who phase-reversed his cortisol pattern completely is represented by the open circles connected by a dotted line on Figure 3. Here he held his basal acrophase position through R_8 and then by R_9 had undergone a rapid 180° phase advance (or delay) which he subsequently held. His Day 8 acrophase was insignificant so that this may have occurred more gradually on non-data days R_{6-7}. However the basal circadian component in his percent cortisol plot (not shown) appeared to be delaying toward sleep on R_{3-4-5} and also diminishing in amplitude, while a late- and post-sleep component was also evident as early as R_3. This lent the R_{3-5} and R_{8-10} cortisol data a bimodal pattern (the latter confirmed by his autocorrelation functions) in which the circadian component (0700) either lost amplitude and dropped out by Day 9 or it was delayed into and masked by early sleep inhibition (Parker et al., in press; Aschoff, 1979; Aschoff, 1960; Aschoff et al., 1975). In either case the late- and post-sleep maximum became the only identifiable acrophase for the cosinor on R_9. After this point the late- and post-sleep component remained as the major unimodal daily component on R_{13-15} (again seen in the autocorrelation function). Thus his reversal was accomplished dramatically and early in the reversal and came about probably by either a true phase delay or a "false" advance that came from identification of a second already existing peak as the new acrophase when the old maximum simply faded out. Clearly it would have been nice to have both D_{6-7} data and unmasked R_{8-9} data here.

To summarize for cortisol, we are convinced there are important events transpiring during the reversal period from which insights about the oscillator(s) involved can be gained, and that this is not just a period of noisy transients. We also strongly suspect that there may be 2 components melded in the basal unimodal circadian pattern, one clearly circadian and the other sleep-related (e.g., a masking and/or a phasing effect) that may become vis-

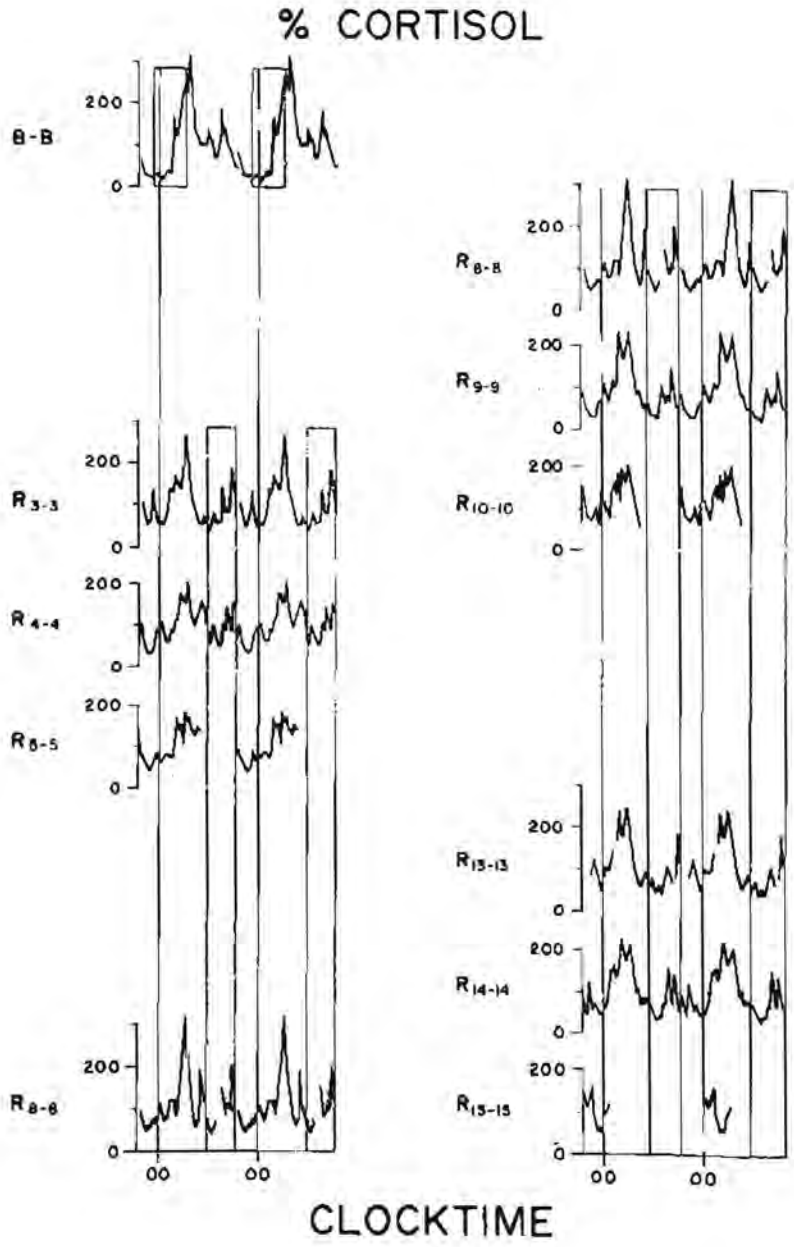

Figure 9. Subject 305's cortisol actigraph across 15 days of sleep-wake reversal. Actigraph details may be found in Figure 1. He has failed to phase-reverse his bimodal daily pattern of maxima by day 15, advancing it only 4-5 hours.

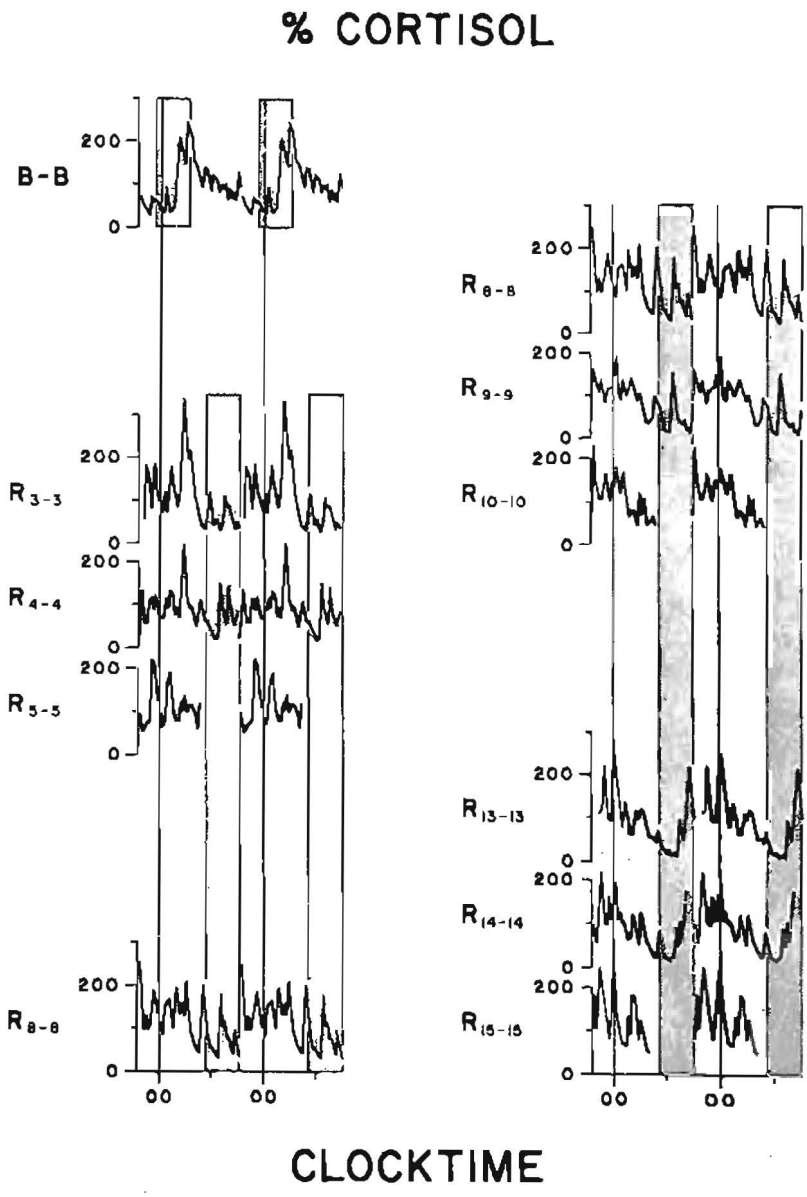

Figure 10. Subject 300's cortisol actigraph across 15 days of sleep-wake reversal. Actigraph details as per Figure 1. He has phase-reversed his unimodal cortisol pattern of maxima by a series of phase-advances across the reversal.

ible during reversal. The circadian component also appears to more commonly phase advance in reversal.

Thyrotropin

The daily maxima are seen to rhythmically recur each evening and to precede the onset of the sleep interval (Parker, Pekary, & Hershman, 1976; Azukizawa, Pekary, Hershman, & Parker, 1976; Weeke, 1973; Weeke, Hansen, & Lundaek, 1975; Alford, Baker, Burger, deKrester, Hudson, Johns, Masterson, Patel, & Rennie, 1973). The rise to peak begins in the early evening and then declines occur across sleep. These results stand in contrast to 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 consistently observed (Webster, Guansing, & Paice, 1972; Nicoloff, Fisher, & Appleman, 1970; Vanhaelst, Van Cauter, Degaute, & Goldstein, 1972; Van Cauter, Leclercq, Vanhaelst, & Goldstein, 1974; Van Cauter, Goldstein, Vanhaelst, & Leclercq, 1975). This contrast indicates sleep to be almost as important a determinant of the locus of maxima of this TSH rhythm as it was for hGH and PRL above (Parker et al., 1976). That sleep exerts an immediate inhibitory effect upon such TSH peaks was shown by advances and delays in sleep during this early evening period from 2000-0000 (Parker et al., 1976). Thus sleep exerts an inhibitory masking effect upon rhythmical daily TSH variation in plasma. In addition, in the absence of this masking effect of nocturnal sleep, the basal daily output of TSH is virtually doubled, an event that should be physiologically significant (Parker et al., in press; Parker et al., 1976). We were also intrigued by the suppressed secretion of TSH that followed on the day after such unmasking when it was accomplished by a 12 hour delay in sleep. At that time (Parker et al., 1976) we were unable to distinguish whether the source of reduced daily TSH release was due to a negative feedback effect of the prior day's huge TSH release or due to a dampening of a circadian rhythm by a phase effect of the reversed sleep-wake cycle. Thus, here we have examined TSH release across more prolonged sleep-wake reversal to gain insight into its apparent circadian rhythmicity and its modulation by sleep-wake cycles.

In Figure 11 are shown the group mean percent TSH concentration data in actigraphic format across the reversal. The basal pattern shows the usual evening pre-sleep peak in TSH whose maximum lies in close relation to sleep onset, and whose waveform is that of a sharp nonsinusoidal peak. On R_1 is seen the effect of acutely unmasking this basal rhythm from inhibitory sleep in darkness. Note that on R_{1-1} the ordinate is 1/2 scale to accommodate the huge release of TSH seen on this night. R_2 shows the suppressed 24 hour pattern of TSH that follows sleep in the 11-19 hour interval. We now know that such suppression also occurs on the third day of serial 6 hour delays in sleep onset, when the previous day's sleep has also been in the 11-2000 hour interval. In this case however, the TSH patterns on the 2 previous days where sleep had occurred in the 05-14 and 11-20 hour segment respectively, were both days in which maximal prolonged TSH secretion had occured (unpublished data). Thus this R_2 pattern of TSH suppression is not the result of negative feedback that we have previously proposed as one alternative explanation (Parker et al., 1976) but is clearly a dampening effect upon phase of the the reversal conditions. R_3 begins the data of our longer sleep-wake study protocol in which subjects now slept in natural light. This is recent preliminary data not yet

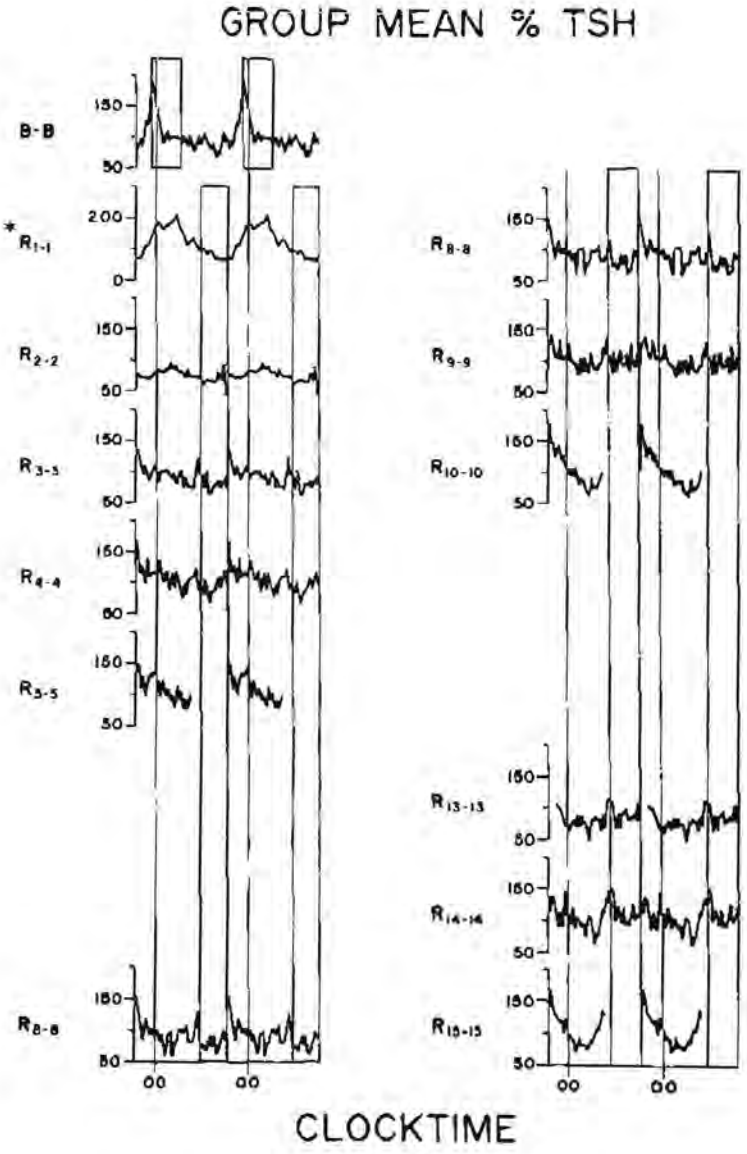

Figure 11. Actigraph of group mean % TSH concentrations across 15 days of sleep-wake reversal. Details as per Figure 1. Note the half-scale ordinate for huge TSH release on R_1.

replicated in a second radioimmunoassay. Here the daily maximum occurs at 1900 hours at the end of sleep - e.g., has advanced from its basal locus at 2300 and has advanced or delayed from its unmasked locus about 04-0600 of R_{1-2}. In addition a small pre-sleep peak in TSH is seen on R_{3-4}. R_{8-10} shows this same pre- and post-sleep pattern though the nadir following the post-sleep peak seems to be advancing. By R_{13-15} the post-sleep component is reduced in amplitude and the pre-sleep component enhanced, so that the phase-reversal of the basal (masked) pattern appears fully established. However, the majority of the advance appears to have been accomplished by a large initial phase advance (or delay) between R_2 and R_3. Here one would have to propose that R_1 sleep or some other phasing event phase delayed the R_2 TSH peak into R_2 sleep where it was masked and subsequently reemerged on R_3. Another alternative is competing synchronizers or uncoupled oscillators responsible for the pre- and post-sleep peak patterns that persist across the study.

In Figure 12 is seen the group mean autocorrelation functions and variance spectra across the reversal for TSH. This shows that the 48 lag (24 hours) peak in positive autocorrelation, which is also seen basally, persists across the reversal except in R_{1-2}. This latter is to be expected from the nature of R_{1-2} TSH release, where the large peak on R_1 is literally not repeated on R_2. In effect, a long delay (> 24 hours or 48 lags) between maxima occurs in the raw data to produce this autocorrelative effect. This is also indicated by the R_{1-2} variance spectra maximum at 0.5 c/d or less.

The group mean TSH acrophases for each segment of the basal and reversal segments and their 95% confidence arcs are shown in Figure 4. Here one sees the initial delay in R_{1-2} attributable largely to unmasking from sleep. Thereafter there is a steady advance of mean ϕ across the reversal. If one assesses the degree of advance from its unmasked locus on R_{1-2} then virtually full 180° phase delay is achieved by R_{8-9}, but if assessed from its basal masked locus to final reversal masked locus, it is only about 8 hours. Note that the R_{8-9} group of TSH acrophases is the only one for all hormones in Figure 4 to fail to achieve significant directionality by Rayleigh testing. This indicates a wide dispersal of acrophases here and speaks to the likelihood that the mode of such shifts is also disparate at this point. A look at the individuals' 24 hour cosinor acrophases in Figure 3 confirms this. Two subjects indeed phase-reversed by a rapid phase advance on R_4 and R_8, and were the same 2 subjects who had fully shifted their cortisol acrophases. The other 2, who had also slowly and incompletely reversed their cortisol acrophases via advances, again did the same for TSH. However, TSH and cortisol were probably not phase-linked as their advances in Figure 3 and Figure 4 were not parallel nor <u>did</u> their group mean cross-correlation functions reveal a fixed-lag peak in cross-correlation to be commonly held across all reversal segments.

Turning to the hormonal actigraphic data of the individuals, one can see in Figure 13 that subject 305 did not reverse completely. Both he and his cohort (311) had basically unimodal patterns across the reversal (and confirmed by their autocorrelation functions) in which the daily peak was a post-sleep one from R_{3-4} on. Neither developed a pre-sleep peak during the reversal (except for 305 on R_3 in Figure 13). Thus both appeared to advance a circadian mechanism a little and then hold. Figure 14 shows an actigraphic example of one subject (300) who did phase-reverse. Both subjects who fully reversed had pre-sleep and post-sleep peaks from R_3 onward and such bimodality was also

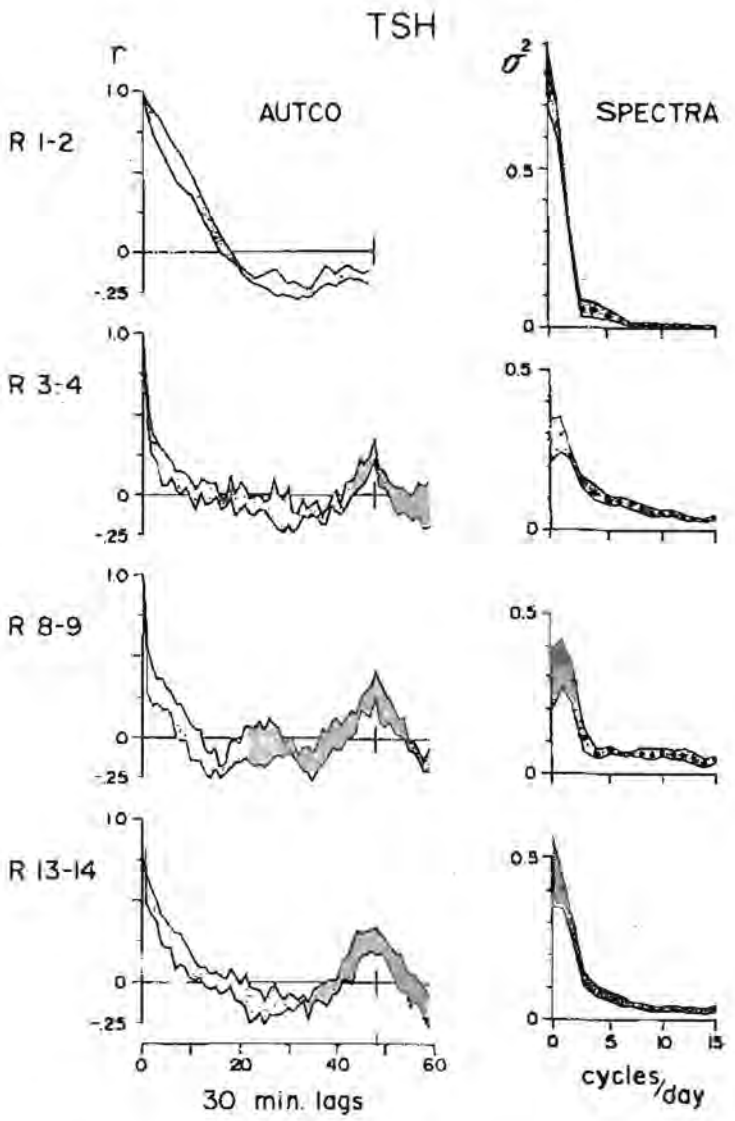

Figure 12. TSH: Group mean autocorrelation (Autco) functions and variance spectra across sleep-wake reversal segments. Details as per Figure 2.

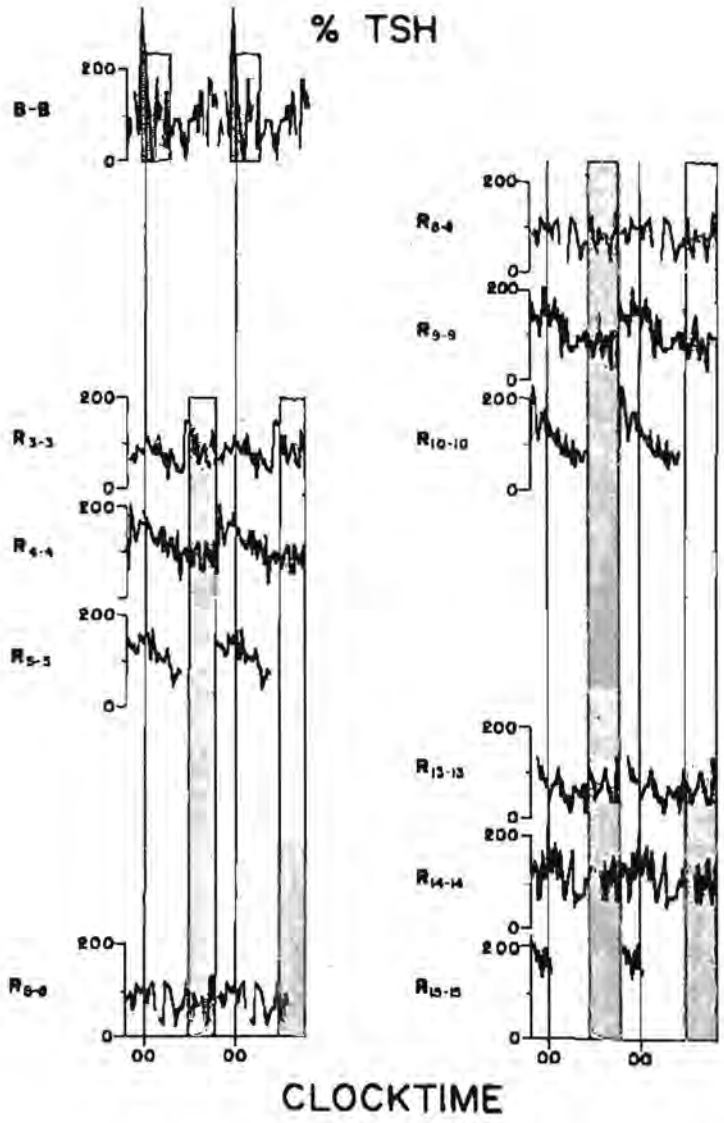

Figure 13. Subject 305's TSH actigraph across 15 days of sleep-wake reversal. Actigraph details as per Figure 1. He fails to fully phase reverse his daily unimodal TSH pattern of maxima, advancing the maxima to 6 hours early in the reversal and then virtually holding in this post-sleep (locus) thereafter.

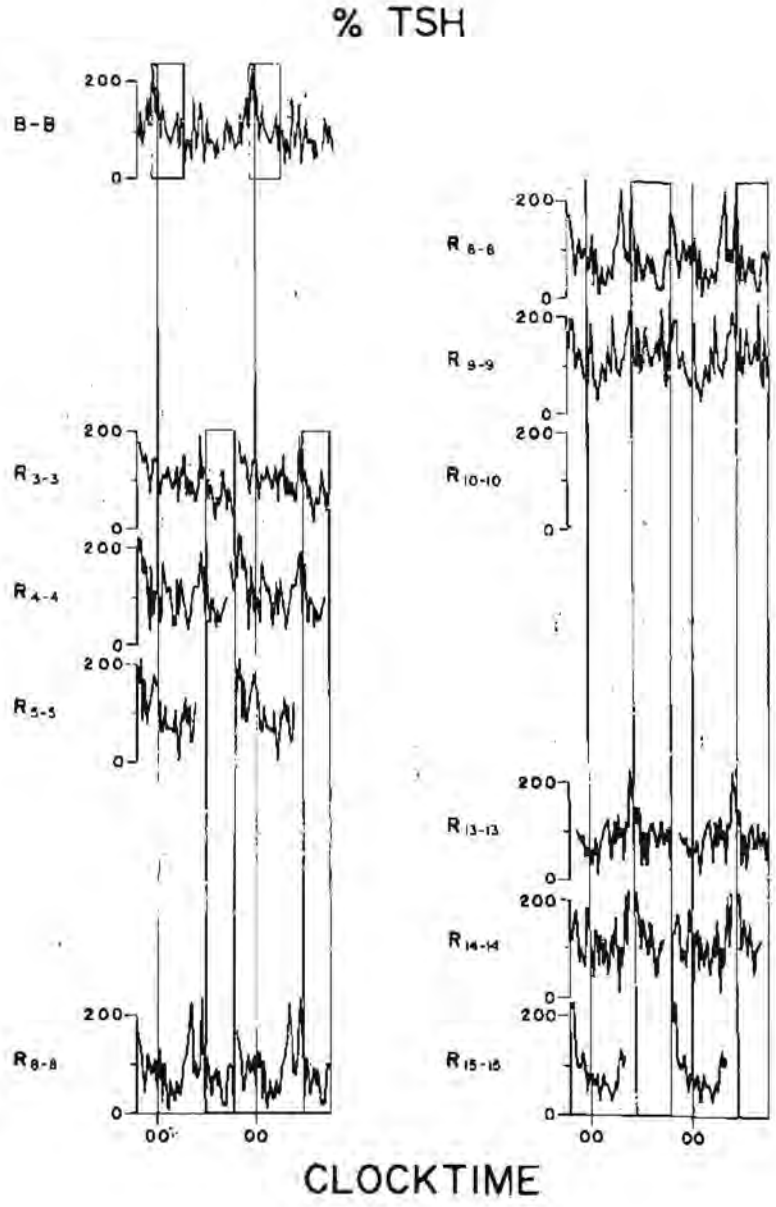

Figure 14. Subject 300's TSH actigraph across 15 days of sleep-wake reversal. Actigraph details as per Figure 1. his daily bimodal pattern of maxima is largely phase reversed by R_3, though a further series of small advances seem to be required to adjust the amplitudes of the pre- post-sleep of R_{3-5}, pre- post-sleep of R_{8-10} to the final pre > post-sleep of R_{13-15} through the sleep-masking effect.

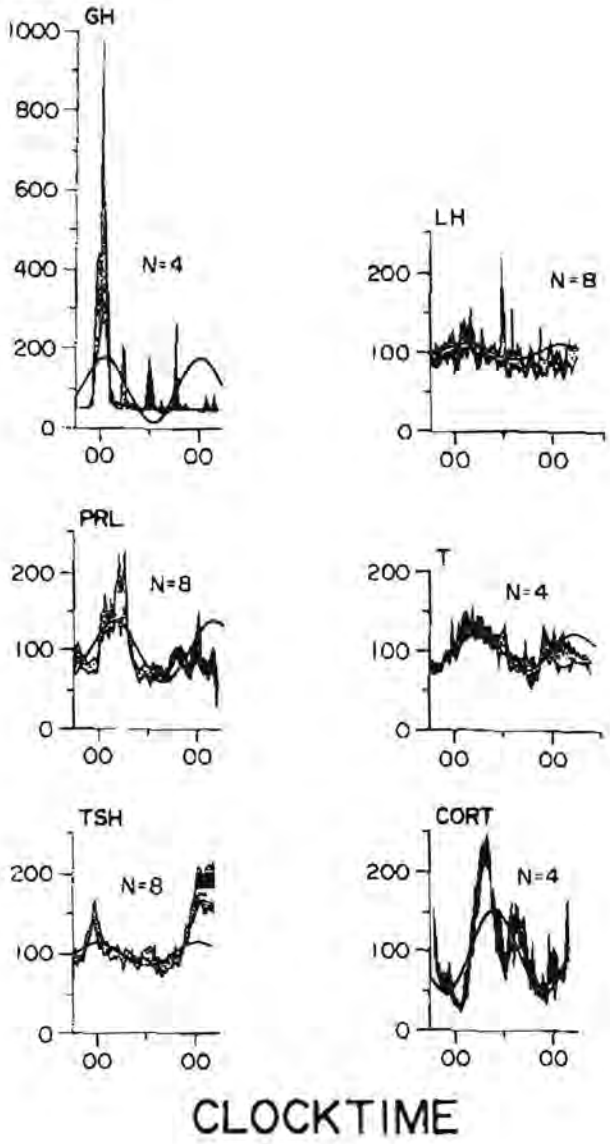

Figure 15. Immediate effect of missed sleep upon overt phasing of hormonal nychtohemeral rhythmicities. The hormonal time series across the first 24 hours of each study for that hormone was fitted with a 24 hour cosine. From these amplitudes (%.C) and acrophases (ϕ), the group mean %C, ϕ, and cosine were derived. This group mean cosine (thick, black, smooth curve) of the fit to the first 24 hours was superimposed on the % 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 a remainder segment on the second night that is devoid of sleep.

seen in their autocorrelation functions. However by R_{13-15} the post-sleep peak and autocorrelation bimodality were fading in 300 and gone in 315, while in both subjects the pre-sleep component had increased in amplitude. This suggests that a circadian mechanism had been phase advanced first into the end of sleep and then had emerged on its "front" side. Sleep unmasking studies are crucial to this formulation and have not yet been done. A rather complex explanation is that there are 2 competing phasers or dissociated oscillators whose effects have been pulled apart in reversal. In any case it's clear that there are circadian, sleep masking and sleep phasing effects occurring here in regard to TSH.

Masking Effects of Sleep

Shown in Figure 15 are pooled data on studies in which basal 23-0700 hours sleep on the first night of study was not followed by sleep at this time on the second night. Superimposed is the group mean cosine derived from first 24 hour fits which is then projected across the second unslept nocturnal segment. For GH, there is no second night peak in the absence of sleep and no cosine fit. For PRL, the only evidence of a circadian residual does not reach the 100% line and the fit deteriorates after 2300 on the unslept night. This further exemplifies our notion of their "circadian" rhythms being almost entirely rhythms of stimulatory masking effects themselves. Cortisol remains immunely circadian in its peaks. However, evidence of its early-sleep inhibitory masking effect is present on the first night and absent on the unslept second. TSH shows its very dramatic unmasking from sleep-related inhibition on the second night. Testosterone and perhaps even young adult male LH also exhibit sleep-stimulatory masking.

This makes it terribly clear that one can't really talk about circadian hormonal acrophases without doing the necessary acute sleep deprivation segments to show where the real acrophase is. From what we have shown here, we are reasonably certain that when this is done, the additional phasing effects of sleeping and waking upon circadian hormonal oscillators may finally become clear to us all.

References

Alford,F.P., Baker,H.W.G., Burger,H.G., deKrester,D.M., Hudson,B., Johns,M.W., Masterson,J.P., Patel,Y.C., & Rennie, G.C. Temporal patterns of integrated plasma hormone levels during sleep and wakefulness. I. Thyroid-stimulating hormone, growth hormone and cortisol. J. Clin. Endocrinol. Metab., 1973, 37, 841-847.

Aschoff,J. Exogenous and endogenous components in circadian rhythms. Cold Springs Harbor Symp. Quant. Biol., 1960, 25, 11-28.

Aschoff,J. Circadian rhythms: General features and endocrinologic aspects. In D. Krieger (Ed.), Endocrine rhythms. New York: Raven Press, 1979, 1-61.

Aschoff,J., Fatronska,M., Gisdke,H., Doerr,P., Stamm,D., & Wisser,H. Human circadian rhythms in continuous darkness: Entrainment by social cues. Science, 1971, 171, 213-215.

Aschoff,J., Hoffman,K., Pohl,H., & Wever,R. Re-entrainment of circadian rhythms after phase-shifts of the zeitgeber. Chronobiologia, 1975,II, 23-78.

Azukizawa,M., Pekary,A.E., Hershman,J.M., & Parker,D.C. Plasma thyrotropin, thyroxine, and triiodothyronine relationships in man. J. Clin. Endocrinol. Metab., 1976, 43, 533-542.

Batschelet,E. Statistical methods for the analysis of problems in animal orientation and certain biological rhythms. Washington, D.C.: The American Institute of Biological Science, 1965.

Boyar,R.M., Rosenfeld,R.A., Kapen,S., Finkelstein,J.W., Roffwarg,H.P., Weitzman,E.D., & Hellman,L. Simultaneous augmented secretion of luteinizing hormone and testosterone during sleep. J. Clin. Invest., 1974, 54, 609-618.

Copinschi,G., DeLaet,M.H., Brian,J.P., Leclercq,R., L'Hermite,M., Robyn,C., Virasoro,E., & Van Cauter,E. Simultaneous study of cortisol, growth hormone and prolactin nychtohemeral variations in normal and obese subjects. Influence of prolonged fasting in obesity. Clin. Endocrinol., 1978, 9, 15-26.

Gallagher,T.F., Yoshida,K., Roffwarg,H.D., Fukushima,D.K., Weitzman,E.D., & Hellman,L. ACTH and cortisol secretory patterns in man. J. Clin. Endocrinol. Metab., 1973, 36, 1058-1073.

Halberg,F., Johnson,E.A., Nelson,W., Runge,W., & Sothern,R. Autorhythmometry: Procedures for physiologic self-measurements and theory analysis. Physiol. Teach., 1972, 1, 1-11.

Honda,Y., Takahashi,K., Takahashi,S., Azumi,K., Irie,M., Sakuma,M., Tsushima, T., & Shizume,V. Growth hormone secretion during nocturnal sleep in normal subjects. J. Clin. Endocrinol. Metab., 1969, 29, 20-29.

Judd,H.L., Parker,D.C., Siler,T., & Yen,S.S.C. The nocturnal rise of testosterone in pubertal boys. J. Clin. Endocrinol. Metab., 1974, 38, 710.

Judd,H.L., Parker,D.C., & Yen,S.S.C. Sleep-wake patterns of LH and testosterone release in prepubertal boys. J. Clin. Endocrinol. Metab., 1977, 44, 865.

Krieger,D.T. Rhythms in CRF, ACTH and corticosteroids. In D.T. Krieger, Ed. Endocrine rhythms. New York: Raven Press, 1979, 123-142.

Krieger,D.T., Allen,W., Rizzo,F., & Krieger,H.P. Characterization of the normal pattern of plasma corticosteroid levels. J. Clin. Endocrinol. Metab., 1971, 32, 266-284.

Krieger,D.T., Kreuzer,J., & Rizzo,F. Constant light: Effect on circadian patterns and those reversal of steroid and electrolyte levels in man. J. Clin. Endocrinol. Metab., 1969, 29, 1634-1638.

Migeon,C.J., Tyler,F.H., Mahoney,J.P., Angel,A.F., Castle,H., Bliss,E.L., & Samuels,L.T. The diurnal variation of plasma levels and urinay excretion of 17-hydroxycorticosteroids in normal subjects, night workers and blind subjects. J. Clin. Endocrinol. Metab., 1956, 16, 622.

Nicoloff,J.T., Fisher,D.A., & Appleman,Jr.,M.D. The role of glucocorticoids in the regulation of thyroid function in man. J. Clin. Invest., 1970, 49, 1922-1929.

Nokin,J., Vekemans,M., L'Hermite,M., & Robyn,C. Circadian periodicity of serum prolactin concentration in man. Br. Med. J., 1972, 3, 56.

Parker,D.C., Judd,H.L., Rossman,L.G., & Yen,S.S.C. Pubertal sleep-wake patterns of episodic LH, FSH, and testosterone in twin boys. J. Clin. Endocrinol. Metab., 1975, 40, 1099.

Parker,D.C., Mace,J., Gotlin,R., & Rossman,L. Nychtohemeral variation of human growth hormone in plasma. J. Clin. Invest., 1968, 47, 76a.

Parker,D.C., Pekary,A.E., & Hershman,J.M. Effect of normal and reversed sleep-wake cycles upon nychtohemeral rhythmicity of plasma thyrotropin: Evidence suggestive of an inhibitory influence in sleep. J. Clin. Endocrinol. Metab., 1976, 43, 318.

Parker,D.C., & Rossman,L.G. Physiology of human growth hormone release in sleep. In R.O. Scow (Ed.), Edocrinology (Proceedings of the Fourth International Congress of Endocrinology, Washington, D.C., June 18-24, 1972). Amsterdam: Excerpta Medica, 1973.

Parker,D.C., & Rossman,L.G. Sleep-wake cycle and human growth hormone, prolactin and luteinizing hormone. In S. Raiti (Ed.), Advances in human growth hormone research. (Publication No. (NIH) 74-612). Washington,D.C.: DHEW, U.S. Government Printing Office, 1974.

Parker,D.C., Rossman,L.G., Kripke,D.F., Gibson,W., & Wilson,K. Rhythmicities in human growth hormone concentrations in plasma. In D.T. Krieger (Ed.), Endocrine rhythms. New York: Raven Press, 1979, 143-173.

Parker,D.C., Rossman,L.G., Kripke,D.F., Hershman,J.M., Gibson,W., Davis,C., Wilson,K., & Pekary,A.E. Endocrine rhythms across sleep wake cycles in normal young men under basal state. In J. Orem (Ed.), Physiology during sleep. New York: Academic Press, (in press).

Parker,D.C., Rossman,L.G., & Vanderlaan,E.F. Sleep-related nychtohemeral and briefly episodic variation in human plasma prolactin concentrations. J. Clin. Endocrinol. Metab., 1973, 36, 1119.

Parker,D.C., Sassin,J.F., Mace,J.W., Gotlin,R.W., & Rossman,L.G. Human growth hormone release during sleep: Electroencephalographic correlation. J. Clin. Endocrinol. Metab., 1969, 29, 871-874.

Pekary,A.E., Hershman,J.M., & Parlow,A.F. A sensitive and precise radioimmunoassay for human thyroid stimulatory hormone. J. Clin. Endocrinol. Metab., 1975, 41, 676.

Quabbe,H.J., Schilling,E., & Helge,H. Pattern of growth hormone secretion during a 24 hour fast in normal adults. J. Clin. Endocrinol. Metab., 1966, 26, 1173-1177.

Rechtschaffen,A., & Kales,A. A manual of standardized terminology, techniques and scoring system for sleep stages of human subjects. (USPH Publication #204). Washington, D.C.: U.S. Government Printing Office, 1968.

Sassin,J.F., Frantz,A.G., Kapen,S., & Weitzman,E.D. The nocturnal rise of human prolactin is dependent on sleep. J. Clin. Endocrinol. Metab., 1973, 37, 436-440.

Sassin,J.F., Frantz,A.G., Weitzman,E.D., & Kapen,S. Human prolactin: 24 hour pattern with increased release during sleep. Science, 1972, 1205-1207.

Sassin,J.F., Parker,D.C., Mace,J.W., Gotlin,R.W., Johnson,L.C., & Rossman,L.G. Human growth hormone release: Relation to slow wave sleep and sleep-waking cycles. Science, 1969, 165, 513-515.

Takahashi,Y., Kipnis,D.M., & Daughaday,W.H. Growth hormone secretion during sleep. J. Clin. Invest., 1968, 47, 2079-2090.

Tyler,T.H., Migeon,C.J., Florentin,A.A., & Samuels,L.T. The diurnal variation of 17-hydroxycorticosteroid levels in plasma. J. Clin. Endocrinol. Metab., 1954, 14, 774.

Van Cauter,E., Goldstein,J., Vanhaelst,L., & Leclercq,R. Effects of oral contraceptive therapy on the circadian patterns of cortisol and thyrotropin (TSH). Europ. J. Clin. Invest., 1975, 5, 115-121.

Van Cauter,E., Leclercq,R., Vanhaelst,L., & Goldstein,J. Simultaneous study of cortisol and TSH daily variations in normal subjects and patients with hyperadrenalcorticism. J. Clin. Endocrinol. Metab., 1974, 39, 645-652.

Vanhaelst,L., Van Cauter,E., Degaute,J.P., & Goldstein,J. Circadian variations of serum thyrotropin levels in man. J. Clin. Endocrinol. Metab., 1972, 35, 479-482.

Vekemans,M., & Robyn,C. The influence of exogenous estrogen on the circadian periodicity of circulating prolactin in women. J. Clin. Endocrinol. Metab., 1975, 40, 886.

Webster,B.R., Guansing,A.R., & Paice,J.C. Absence of diurnal variation of serum TSH. J. Clin. Endocrinol. Metab., 1972, 34, 899-901.

Weeke,J. Circadian variations of the serum thyrotropin level in normal subjects. Scan. J. Clin. Lab. Invest., 1973, 31, 337-342.

Weeke,J., Hansen,A.P., & Lundaek,K. Inhibition by somatostatin of basal levels of serum thyrotropin (TSH) in normal men. J. Clin. Endocrinol. Metab., 1975, 41, 168-171.

Weitzman,E.D., Goldmacher,D., Kripke,D.F., MacGregor,P., Kream,J., & Hellman,L. Reversal of sleep-waking cycle: Effect on sleep stage pattern and certain neuroendocrine rhythms. Trans. Am. Neurol. Assoc., 1968, 93, 153-157.

Weitzman,E.D., Nogeire,C., Perlow,M., Fukushima,D., Sassin,J., MacGregor,P., Gallagher,T.F., & Hellman,L. Effects of a prolonged 3-hour sleep-wake cycle on sleep stages, plasma cortisol, growth hormone and body temperature in man. J. Clin. Endocrinol. Metab., 1974, 38, 1018-1030.

Weitzman,E.D., Perlow,M., Sassin,J.F., Fukushima,E., Buralk,B., & Hellman,L. Persistency of the 24 hour pattern of episodic cortisol secretion and growth hormone release in blind subjects. Trans. Am. Neurol. Assoc., 1972, 97, 197-199.

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EDITORS

Laverne C. Johnson
Donald I. Tepas
W. P. Colquhoun
Michael J. Colligan

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Centers for Disease Control
National Institute for Occupational Safety and Health
Division of Biomedical and Behavioral Science
Cincinnati, Ohio 45226

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