

ENTRAINMENT OF HUMAN CIRCADIAN RHYTHMS BY LIGHT-DARK CYCLES: A REASSESSMENT

CHARLES A. CZEISLER,* GARY S. RICHARDSON, JANET C. ZIMMERMAN,
 MARTIN C. MOORE-EDE† and ELLIOT D. WEITZMAN

Laboratory of Human Chronophysiology, Department of Neurology, Montefiore Hospital and
 Medical Center and the Albert Einstein College of Medicine, Bronx, NY 10467
 and †Department of Physiology, Harvard Medical School, Boston, MA 02115, USA

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Abstract—Man is the only eukaryotic organism in which it has been reported that the circadian system cannot be entrained to a 24-h period by a simple light-dark (LD) cycle. In this paper, we reexamine the evidence for that claim and demonstrate that there were some fundamental flaws in the experimental design of the previous studies on which this conclusion was based. We report new studies in which we tested the efficacy of LD cycles in entraining the circadian rhythms of human subjects living in isolation from environmental time cues. We found that the cyclic alternation of light and dark, when applied to human subjects in a comparable way to experiments in other species, was an effective entraining agent. Our results and a critical review of the literature indicate that a LD cycle alone can be an effective environmental synchronizer of the human circadian timing system. Other factors, such as the knowledge of time of day, social contacts, the feeding schedule, and the imposed rest-activity schedule may contribute to stable entrainment, although their relative strengths as synchronizers have yet to be determined.

INTRODUCTION

Since DeCandolle's original observation (1832) that the period length of the daily leaf position rhythm of plants placed in continuous light was shorter than 24 h, the observation that such free-running self-sustained oscillations (circadian rhythms) persist with a non-24-h period in the absence of periodic environmental inputs has become well established. In the early 1960s, two groups independently sought to verify this phenomenon in man. Aschoff and Wever (1962) placed subjects singly within a sealed cellar below a Munich hospital for 8–19 days and Siffre (1964) lived by himself in an underground cavern for two months in 1962. Both groups reported that the spontaneous or free-running period length of the human rest-activity cycle was *greater* than 24 h, averaging 25 h. Thus, it was clear that under natural conditions certain periodic environmental stimuli (called zeitgebers by Aschoff, 1960) were synchronizing or entraining the human rest-activity cycle to a period of exactly 24 h.

Information regarding the nature of such periodic environmental stimuli can be obtained by examining the experimental conditions common to the subjects in both of those free-running experiments. They included: ignorance of the time of day; self-selection of ambient light level; isolation from social contact; an unscheduled regimen of eating; an unscheduled regimen of bedrest and activity. Thus, any one or more of a number of periodic factors, which we have summarized in Table 1, could be responsible for normally

entraining human circadian rhythms to the 24-h period of the earth's rotation.

Aschoff and Wever have concluded from their extensive experiments during the past 15 years that 'social cues' (Factor III) were responsible for the normal entrainment of human circadian rhythms. They reported that the LD cycle (Factor II) was too weak a zeitgeber to entrain the circadian rhythms of man (Wever, 1970; Aschoff *et al.*, 1971a; Wever, 1974; Aschoff and Wever, 1976). Since those conclusions set man apart from all other organisms previously studied, and since their concept of "social cues" was not fully defined, we have reviewed the published studies.

PREVIOUS STUDIES: A REINTERPRETATION

Knowledge of time (Factor I). In 1964, Mills reported that the knowledge of time (Factor I) was insufficient to entrain the circadian rhythms of a man who had a watch while he lived in a cave, isolated from human contact for 3 months. Findley *et al.* (1963) confirmed that finding with a different subject. However, careful analysis of the free-running patterns

Table 1. Possible factors responsible for entrainment of human circadian rhythms

Factor
I. Knowledge of the time of day
II. Environmental light-dark cycle
III. Social contacts
IV. Timing of food availability
V. Scheduling of bedrest and activity

*To whom correspondence should be addressed.

of both of those subjects suggested that the knowledge of time may have modulated their periodic behavior, revealing typical patterns of 'relative coordination' to an external zeitgeber too weak to entrain an endogenous rhythm (von Holst, 1939; Enright, 1965; Aschoff, 1965; Saunders, 1977). The extent of that influence varied between and within each of those experiments. The knowledge of time (Factor I) is therefore probably a weak zeitgeber of human circadian rhythms, which cannot account for the normal entrainment of human circadian rhythms to a 24-h day.

Light-dark cycles (Factor II). Cycles of alternating light and darkness (LD cycles)[‡] are the most powerful environmental synchronizers of biological rhythms in a wide variety of organisms from unicellular algae to higher mammals (Bruce, 1960; Pittendrigh, 1960; Zucker, 1976; Moore-Ede and Sulzman, 1977), including primates (Hoffman *et al.*, 1969; Rohles and Osbaldiston, 1969; McNew *et al.*, 1972; Moore-Ede *et al.*, 1977). Aschoff and Wever presumed initially that LD cycles were important synchronizers in man and therefore designed their facility in Andechs, Germany, to allow for extensive manipulation of illumination conditions (R. Wever, personal communication). Free-running circadian rhythms were observed with both self-selected LD cycles and constant light of varying intensity (Wever, 1969). Imposed LD cycles (Factor II) were initially reported to be a very effective zeitgeber to entrain those free-running rhythms in man (Aschoff *et al.*, 1969).

By chance, however, in one study an LD cycle entrainment experiment was conducted without the usual use of the system of gong sounds which signalled and/or awakened subjects for periodic urine collections§. The investigators were surprised to find that an imposed LD cycle without the periodic gong signals was insufficient to entrain the subject to 24-h day (Wever, 1970). Entrainment occurred only when the LD cycle and gong signals were paired.

This led Wever to conclude that a simple light-dark cycle (Factor II), complete with dawn/dusk transitions, was a weak zeitgeber in man. Figure 1 shows the rest-activity pattern from such an experiment double-plotted, in which the rest-activity cycle apparently failed to entrain to a 24-h LD cycle. Wever did note relative coordination of the rest-activity cycle pattern to the 24-h LD cycle in some subjects. The gong signal was interpreted as a personal call from the experimenter and therefore a "social" contact (Factor III), making the gong signal a much more powerful zeitgeber than the LD cycle (Wever, 1970;

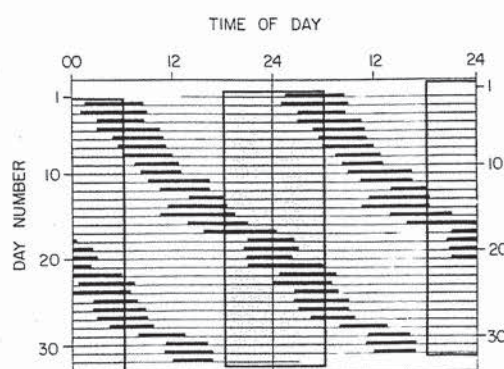


Figure 1. Bedrest-activity cycle in subject living in an environment without knowledge of time. Data are double-plotted in a raster format, with successive days plotted beneath each other as well as side by side to aid visualization. Thin horizontal lines indicate reported waking hours; reported bedrest episodes indicated by heavy black horizontal bars. Imposed cycle of overhead illumination is shown, with stippling indicating no overhead light. However, subject was allowed the use of kitchen, bathroom, bedside and desk lamps at self-selected times (Redrawn from Wever, 1970).

Wever, 1974). However, the efficacy of the gong signal as a principal zeitgeber (i.e. in the absence of an LD cycle) was never evaluated. Nonetheless, those experiments have led to a general conclusion that LD cycles are a weak zeitgeber in man.

A critical examination of the experimental conditions in which those human LD entrainment studies were conducted indicated that the experimenters permitted the subjects to use kitchen, bathroom, bedside and desk lamps as sources of self-selected light during the 'dark' phase of each cycle. Those tests of light-dark cycle entrainment were, therefore, not comparable to those carried out in other organisms, being more similar to experiments with self-selected LD cycles. It has been repeatedly shown that both animals (Wahlström, 1965; Aschoff *et al.*, 1971b; Cone, 1974; Warden and Sachs, 1974) and humans (Aschoff and Wever, 1962; Siffre, 1964; Colin *et al.*, 1968; Wever, 1969; Czeisler, 1978; Weitzman *et al.*, 1979) will free-run under conditions of self-selected LD. We, therefore, do not feel that one can conclude that an LD cycle is an ineffective zeitgeber in man (Factor II).

Social contacts (Factor III). Aschoff and Wever concluded from the above experiments that 'social cues' (Factor III) were by far the most important zeitgeber in man. However, that conclusion presumes that the subjects perceived only the automatic gong signal and not the automatic overhead light switch as a personal call from the experimenter and that their circadian system responded to that perceived "social contact." An alternative argument can be made that the regular gong signals and the accompanying performance tests, which were timed to occur more frequently during expected waking than sleeping hours (as well as at bedtime and waketime) acted as an alarm clock which interrupted the subject's sleep each

[‡]Abbreviations: DSPS, delayed sleep phase syndrome; EF, eating-fasting; LD, light-dark; RHT, retinohypothalamic tract; SCN, suprachiasmatic nuclei.

[§]This experiment was not initially performed to test the influence of the gong signals, which they had presumed to be insignificant. Mechanical failure of the gong signal system at the beginning of one experiment led to the discovery of their influence (Wever, 1979, p. 151).

morning, thereby facilitating his adaptation to the imposed cycle of overhead lighting^{||}. The latter interpretation would support a role for Factor V, the scheduled regimen of bedrest and activity, as important in entrainment.

Aschoff *et al.* (1971a) reported that there was no change in either the period length or the waveshape of multiple circadian rhythms in subjects studied for four days in an LD cycle as compared to four days in constant darkness, provided they maintained the subjects on a "rigorous schedule, with rest time in bed from 23.30 to 07.30 hours" (Aschoff *et al.*, 1971a). Urine collection, performance testing and mealtimes were similarly scheduled. They concluded that "social cues apparently were sufficient to entrain human circadian rhythms" (Aschoff *et al.*, 1971a). As in the case of the gong signals, the specific nature of the 'social cues' involved were not precisely defined. The only reference to the social conditions mentioned in the paper was that the subjects were housed in pairs. It was not clear whether those authors felt that the social cues had to be periodic for effective entrainment, or whether mere interaction with an entrained individual was sufficient. Wever (1979) later indicated that every 3 h (including awakenings from sleep) the subjects gave urine samples and performed tests "... in the presence of an experimenter." There were also gong signals every 3–4 h.

Other evidence cited for the importance of social contacts as a zeitgeber in man has been the mutual entrainment which usually occurs among members of a group living together in isolation from time cues (Pöppel, 1968; Wever, 1974; Winget and Leach, 1977). However, interpretation of those studies is confounded by the fact that such subjects might have tended to schedule their activities, such as mealtimes, bedrest and activity times, around the periodic behaviors of each other and thereby be mutually entrained via Factors IV and V. Whether periodic social contacts (in the absence of concurrent behavioral scheduling) would act as an entraining agent has not been tested in man; Sulzman *et al.* (1977b) have shown the periodic interaction between squirrel monkeys to be an ineffective zeitgeber in that primate species, whereas the presence of entrained, freely moving bats is apparently sufficient to entrain other bats caged deep within a cave (Marimuthu and Chandrashekar, 1979).

Timing of food availability (Factor IV). Since the first report of meal timing as an effective zeitgeber in rodents by Curt Richter in his Ph.D. thesis published in 1922, there have been many reports suggesting that food can act as a zeitgeber. More recently, the zeitgeber action of near-24-h cycles of eating and fasting (EF cycles) has been rigorously demonstrated in non-

human primates by Sulzman *et al.* (1977a,b, 1978) and rodents (Boulos and Terman, 1981). In those studies, EF cycles were shown to entrain multiple behavioral and physiological rhythms according to accepted criteria for entrainment. However, there have been few satisfactory studies to determine if meal timing acts as a zeitgeber in man. A recent study (Goetz *et al.*, 1976) which examined restricted feeding schedules is uninterpretable because they did not remove other potential zeitgebers from the environment. This question, therefore, deserves further study.

Scheduling of bedrest and activity (Factor V). The importance of regularly scheduled bedrest and activity times (Factor V) unfortunately cannot be separated from that of social contacts (Factor III) in Wever's experiments. Webb and Agnew (1974) recognized this problem and postulated that "the force of the entrainment was in the regularity of the behavioral scheduling rather than the social cues." However, in their experiments, they also did not separate the influence of the behavioral scheduling, social contacts, mealtimes or LD cycles, since they were always scheduled concurrently. The subjects knew the time of the scheduled bedrest/dark episodes and were sent a note by the experimenter just prior to each "lights off" requesting them to try to sleep. Thus, although they concluded that a rigid schedule of bedrests *alone* was capable of entraining human circadian rhythms, they ignored the influence of the LD cycle, structured social contact and the knowledge of time (Factors I, II and III) which were also part of their entrainment stimulus.

STUDIES AT THE MONTEFIORE HOSPITAL LABORATORY OF HUMAN CHRONOPHYSIOLOGY

Methods. In order to determine the strength of a simple LD cycle as an entraining agent in man, we imposed LD cycles on two subjects (CA and DB; male, ages 23 and 24). The subjects were told that the lights might go on or off at any time, whether convenient or not. The subjects lived in a specially constructed three-room experimental apartment suite, part of the Laboratory of Human Chronophysiology at the Montefiore Hospital and Medical Center described in detail elsewhere (Czeisler, 1978; Weitzman *et al.*, 1979). The environment was free of all obvious time cues throughout the experimental period. There were no information sources for time (e.g. no natural sunlight, clocks, radio, television, current newspapers, etc.) in the apartment. No attempt was made to shield the apartment from subtle environmental periodicities such as daily changes in levels of humidity, barometric pressure, etc. Infrared light sources remained on continuously throughout the studies to allow video monitoring during the "lights out" period, when the subjects were in total darkness (i.e. no visible light). Subjects were not isolated from social contact with other people. Members of the staff would frequently enter the apartment to collect a biologic specimen or talk with the subject (normally less than 5 min). The subject could speak with a staff member via intercom at any time of his choosing. Work shifts of laboratory personnel who interacted with the subject were scheduled at random times and for varied durations (3 to 8 h in length). All staff were instructed and coached to avoid communication regarding the date or time of day to the subject.

^{||}In his recent monograph on these experiments, Wever even reported that "... the regular signals helped the subjects schedule their days. Some subjects numbered the signals as 'day gongs 1 to 6' and the 'nightgong'" (Wever, 1979, p. 151).

Table 2. Summary of Factors controlled under each experimental condition*

Factor		Fully scheduled regimen	Unscheduled regimen	Scheduled regimen LD cycle†
I	Knowledge of the time of day	Self-estimated	Self-estimated	Self-estimated
II	Environmental LD cycle	Scheduled‡	Linked to chosen wake times	Scheduled
III	Social contacts§	Scheduled	Linked to chosen wake times	Linked to chosen wake times
IV	Timing of food availability	Scheduled‡	Self-selected	Self-selected
V	Scheduling of bedrest and activity	Scheduled‡	Self-selected	Self-selected

* Ambient temperature was self-selected throughout these studies.

† Scheduled (by the experimenter) according to the subject's previously reported daily diary.

‡ The light and dark episodes were separated by 1 h dawn-dusk (twilight) transitions.

§ Subjects were not disturbed or visited by staff for any reason during chosen or scheduled bedrests unless subject called for aid (e.g. for nocturnal micturition).

The subjects were studied in the environment under three different conditions: fully scheduled regimen (scheduled bedrest/dark, activity/light, mealtimes and social interactions; $T = 24$ h), unscheduled regimen (self-selected bedrest-activity, meal and LD times); and the scheduled LD cycle regimen (with twilight transitions; $T = 24$ h). The particulars of each condition are summarized in Table 2.

Fully scheduled regimen. The lengths of the scheduled bedrest/dark and activity/light times were based on an average of the times recorded by the subject in his sleep-wake log kept for several weeks just prior to his participation in the study. This was done in order to accommodate normal interindividual variations in sleep length (Webb, 1970). During the fully scheduled regimen, (days 1-5), the subjects were scheduled to go to bed and to arise from bed exactly in phase with their previously-reported average habitual bedtimes and waketimes (calculated from the prior sleep-wake log). The subjects were not aware of the clocktimes of the imposed schedule. During this imposed bedrest-activity schedule, the lights were turned off at the beginning and turned on at the end of each bedrest episode. Light-dark transitions were abrupt, except for a 5 min period of adaptation to dim light allowed at wake-time. The subject's meals were served to him on a schedule which was in keeping with his previously-reported daily pattern. Non-scheduled snacks were also permitted.

Unscheduled regimen. Before bedtime on the last fully scheduled bedrest (Bedrest No. 5), the subject was told that until further notice, his activities would no longer be scheduled. He was told that it would be his decision as to when he wanted to get up or go to bed, but that he could not control the level of ambient lighting. Subjects were asked to go to bed when they felt tired and to wake up when they felt rested. Whenever the subject did request to go to sleep, the lights were turned out at the beginning of bedrest and turned on again when the subject requested to get out of bed. Thus, during this section of the experiment, the subjects were studied on a self-selected feeding, LD, bedrest-activity and social interaction regimen.

Scheduled light-dark cycle regimen. A periodic light-dark cycle was imposed while the subjects were free-running. No special notice was given to the subject at the start of the

scheduled LD protocol, since the instructions (to go to bed when tired and to get up from bed when rested) remained unchanged*. The subjects were unaware of the temporal characteristics of that imposed LD cycle. Light-dark transitions were accomplished gradually over 1 h, by using a Dawn-Dusk Simulator which consisted of a Harvard Infusion-Withdrawal Pump (Harvard Apparatus Company, Dover, MA) mechanically interfaced to a pair of solid state linear stroke dimmers (Lutron Company, Harrisburg, PA) wired in series with the overhead room and hall lights.

As in the unscheduled regimen, subjects continued to self-select the timing of feeding, bedrest-activity and social interaction. Specifically, the subjects were never disturbed during the bedrest episode, whether the lights were on or not. During self-selected waketimes, even when it was dark, the subjects interacted with the technicians for the usual physiologic and behavioral monitoring.

The LD schedule for subject CA provided 16.5 h of light (including an hour of both dawn and dusk transitions) and 7.5 h of darkness; for subject DB, there were 16 h of light and 8 h of darkness (with the same transitions). Three tests of entrainment to a light-dark cycle were performed. In the first (LD Trial I), an LD cycle was imposed on subject CA after 11 wk of self-selected LD and bedrest-activity cycles. Two LD entrainment trials were conducted with subject DB, the first (LD Trial II) after 4 wk of the unscheduled regimen and the second (LD Trial III) a week after Trial II had ended and the subject had returned to the unscheduled conditions. Trial I was 14 days long, Trial II 19 days and Trial III 9 days long. Each LD entrainment trial was followed by a return to the unscheduled condition with self-selected LD, mealtimes and bedrest-activity cycles for 5-9 days.

RESULTS

Effects of an imposed LD cycle

Both subjects demonstrated typical non-24-h free-running period lengths during the protocol consisting of self-selected LD cycles. However, when the LD cycle was imposed, the free-running rhythm of the self-selected bedrest-activity cycle synchronized to the LD cycle for each of the three experimental trials. Sleep request lagged the onset of darkness by an average of 103 min. Figure 2 shows the bedrest-activity

*Before the start of the experiment, however, the subjects were told that they could not control the ambient lighting, and specifically that it might be turned on or off at undesired times. They each consented to this beforehand.

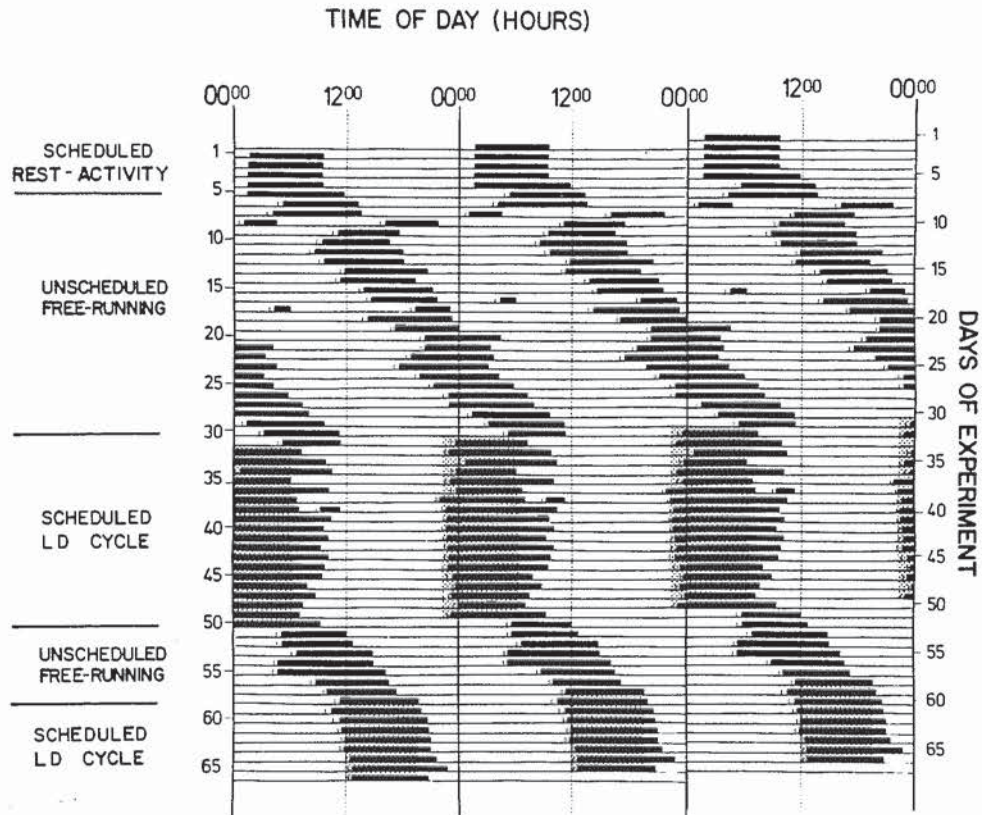


Figure 2. Triple plotted bedrest-activity cycle in subject DB living in an environment without the knowledge of time. Symbols as in Fig. 1. Thin vertical hatch marks indicate the beginning of bedtime preparation before unscheduled bedrests. A strict LD cycle was imposed for two different segments, as shown on left. Conditions labeled on left are defined in Table 2 and in text.

cycle in one of the subjects (DB) who was twice subjected to an LD cycle.

All other rhythms documented in these subjects were entrained to the imposed LD cycle, including the core body temperature cycle and the polygraphically-recorded sleep-wake cycle. Sometimes, however, several cycles of transients were seen before entrainment was evident. For example, a number of cycles were required before the waveshape of the core body temperature rhythm changed to that typically seen during entrainment to a 24-h day. Figure 3 demonstrates the phase relationship and waveshape of the core temperature cycle (left panels) during various conditions in the other subject (CA). His bedrest-activity cycle is shown in the right panels. In that figure, left panels (a) and (b) show the temperature rhythms recorded during the scheduled baseline and the unscheduled free-run. It is clear that the waveshape of the free-running body temperature cycle (3b) differs from that of the entrained cycle (3a). In addition, the phase relationship of the free-running temperature cycle to the bedrest-activity cycle is different from their phase relationship in the entrained state. On fully scheduled baseline days (3a), bedrest onset occurs when the core temperature is high and is followed by a steep drop of core temperature making the pattern resemble a square wave; on unscheduled days (3b), bedrest onset

occurs at or near the lowest values of core temperature, which then rises during sleep. This latter pattern persists at the beginning of the LD entrainment section (3c), but following several transient cycles, the original "square wave" shape characteristic of entrainment reappears (3d). This was then followed by a return to the free-running waveshape and phase relations, within 36 h after release from LD (3e).

When the subjects were allowed to resume self-selected LD cycles, they again had a free-running rhythm. The phase from which the rest-activity cycle resumed its free-run was determined by the phase position of the previously imposed LD cycle, rather than the phase predicted by the previous free-running segment (e.g. days 10-30 in Fig. 2). In fact, after release from the first entraining LD cycle, free-running bedrest onsets in subject DB (days 51-57, Fig. 2) occurring at the phase waketines would have been predicted based on the prior free-run. This confirms that the imposed LD cycle acted as an effective entraining agent for the human circadian system, rather than eliciting a masking effect.

DISCUSSION

The results of this study challenge the often cited conclusion that an artificial light-dark cycle is a weak

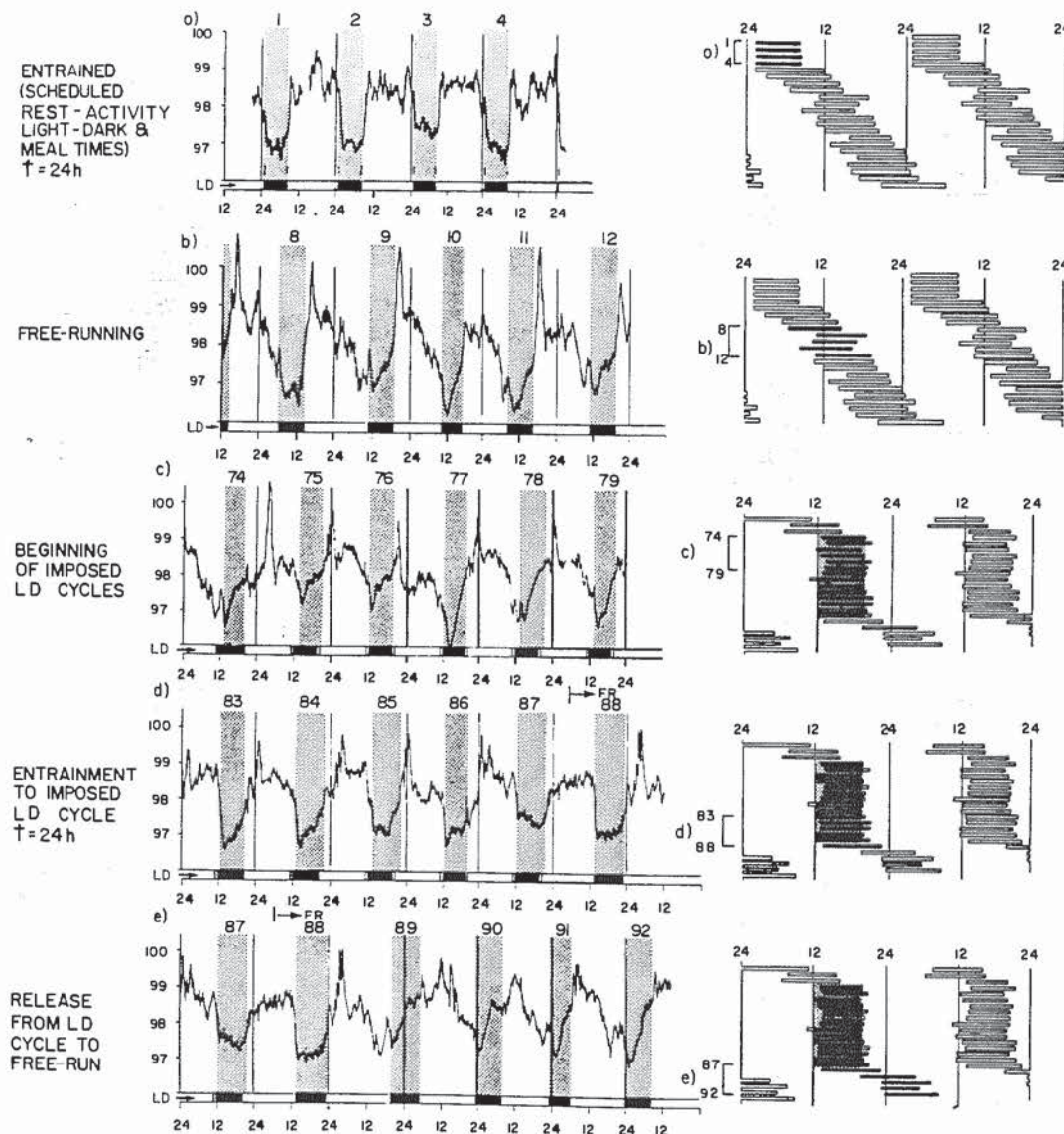


Figure 3. Core body temperature ($^{\circ}\text{F}$) in subject CA under various conditions (see text). Double-plots of bedrest episodes in right-hand panels correspond to the days of temperature recording shown in the left-hand panels, respectively, with bedrest episodes on those days blackened in at right for ease in identification. Time of bedrest episodes indicated by stippling in the left-hand panels, with dark episodes indicated by black bars below each temperature plot. As in Figs. 1 and 2, imposed LD cycle illustrated by stippling of dark times in right-hand panels.

zeitgeber in man. Both the bedrest-activity and body temperature rhythms, which can each free-run with a different average period within the same individual (Wever, 1979; Czeisler *et al.*, 1980a,b), were successfully entrained by the imposed LD cycle. Whether this entrainment occurred because of the influence of darkness on the subjects' decision to go to sleep (a scheduling effect—Factor V) or whether it was due to some more direct action of the LD changes themselves (Factor II) on the circadian timing system can-

not be determined from these studies. Nonetheless, it is clear that the response of the human circadian system to a periodic LD cycle is *not* different from that of other mammals.

Social contact *per se* does not appear to act as an effective zeitgeber. Typical free-running patterns were observed in subjects on self-selected LD cycles living without external time cues who had frequent *ad lib.* contact with personnel who knew the time of day[¶], but did not impart this information to the subject (Czeisler, 1978). Reinterpretation of the previously published data (Aschoff *et al.*, 1971a) in relation to these results strongly suggests that it was not the social contact *per se* which entrained the circadian

[¶]The schedule of social contacts with any individual staff member was aperiodic, in that staffers randomly assumed shifts of random duration. The times of social interaction were generally self-selected by the subject.

rhythms of subjects studied in constant darkness, but rather the secondary consequences of the scheduling of the bedrest-activity and/or meal times (Factors IV or V). Both Factors IV or V could account for the mutual entrainment usually observed between members of a group who live together in isolation of time cues (Pöppel, 1968; Wever, 1974; Winget and Leach, 1977). It is probable that such subjects will schedule their daily behavioral activities, such as mealtimes, and sleep and wake times, around the periodic behaviors of each other and thereby mutually entrain each other via Factors IV and V. In our experiments, the social contact was not periodic, but rather self-selected, i.e. available whenever the subject wanted. It is clear from our results that self-selected social contacts (Factor III) devoid of knowledge of time (Factor I) are not a strong zeitgeber of humans. Whether periodic social contacts (in the absence of concurrent behavioral scheduling) could act as an entraining agent has not been tested in man.

The morphological basis for circadian entrainment by light has not been determined in man. In other mammals, including non-human primates (Moore, 1978; Moore-Ede *et al.*, 1980b), a retinohypothalamic tract (RHT), originating in the retina, monosynaptically innervates a key circadian pacemaker, the suprachiasmatic nuclei (SCN) of the hypothalamus (Rusak and Zucker, 1979). The existence of an RHT in man, however, cannot be verified by the autoradiographic techniques currently used in animal studies because these require pre-mortem administration of tritiated material. However, we have identified a neuronal cluster apparently homologous to the SCN in human brains (Lydic *et al.*, 1980). More indirect approaches, including mathematical modeling of the human circadian system (Kronauer *et al.*, 1981) and physiological studies in non-human primates after suprachiasmatic lesions (Moore-Ede *et al.*, 1980a), provide further evidence that there is a circadian pacemaker homologous to the SCN in man, that this pacemaker predominantly drives rest-activity cycles in primates, and that it is directly coupled to zeitgeber inputs such as the LD cycle.

The findings of this study are potentially important for the understanding of the pathophysiology of disorders of the entrainment mechanism in man, including the hypernycthemeral sleep-wake syndrome (Elliott *et al.*, 1971; Miles *et al.*, 1977; Kokkoris *et al.*, 1978; Weber *et al.*, 1980; T. Wehr, personal communication; L. Miles, personal communication). Long-term records utilizing ambulatory temperature and activity monitoring devices have demonstrated that these individuals maintain non-24 h rhythms while living in society, being unable to entrain to a 24-h day. Another much more common disorder of the entrainment mechanism is the delayed sleep phase syndrome (DSPS), characterized by sleep onset insomnia and difficulty in morning awakening (Weitzman *et al.*, 1981). Our hypothesis of an inadequate capacity of the circadian timing system to phase

advance in such patients was supported by our recent success in their treatment, by a technique based on that model of DSPS pathophysiology (Czeisler *et al.*, 1981).

It is possible that the availability of electric lights has contributed to the pathogenesis and maintenance of these entrainment disorders by enabling self-selection of the LD cycles. Whether an impaired reception of visual inputs, or their transmission to circadian pacemakers, explains the reduced capability to phase advance in DSPS patients is unknown, since none of the patients reported has been studied in a strict LD cycle. However, the demonstration of one blind man in the hypernycthemeral group who was unable to entrain to a 24-h day (Miles *et al.*, 1977) supports the notion that LD changes may play a direct role in synchronization of circadian rhythms in man, quite apart from their influence on scheduling. Furthermore, a more recent study with the same blind patient demonstrated failure of entrainment despite the 4-6 months of imposition and documented adherence to a strict 24 h sleep-wake schedule (Nafarrate and Miles, 1981). This indicates that Factor IV alone was insufficient for entrainment in the absence of visual inputs (Factor II).

That finding certainly supports the conclusion that the LD cycle may have a direct synchronizing effect on the human circadian timing system, in a manner which may be similar to that suggested by the work of Moore and Klein (1974) in other mammals. Identification of the LD cycle as an effective zeitgeber provides an experimentally reproducible probe to investigate the capacity of the human circadian system to phase shift, in both normal subjects and patients. The number of cycles required to establish a stable phase relationship between the LD, sleep-wake and temperature cycles may be a function of the free-running phase position at which the LD cycle is imposed. This would be similar to the differences observed in the rate of adjustment to jet-lag following eastward compared to westward flights (Klein *et al.*, 1977). The duration of the transition time required will probably vary with the direction and degree of shift required to adapt to the imposed cycle (Mills *et al.*, 1978). Taken together, these studies may therefore not only contribute to the understanding of physiological systems controlling entrainment, but also to the evaluation and treatment of the sighted patients described above who are disabled by their inability to entrain to a 24-h day; such individuals may benefit from the imposition of a strict LD cycle regimen.

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REFERENCES

- Aschoff, J. (1960) *Cold Spring Harbor Symp. Quant. Biol.* **25**, 11–28.
- Aschoff, J. (1965) In *Circadian Clocks* (Edited by J. Aschoff), pp. 95–111. North-Holland, Amsterdam.
- Aschoff, J., M. Fatranská, H. Giedke, P. Doerr, D. Stamm and H. Wisser (1971a) *Science* **171**, 213–215.
- Aschoff, J., U. Gerecke, A. Kureck, H. Pohl, P. Reiger, U. v. Saint-Paul and R. Wever (1971b) In *Biochronometry* (Edited by M. Menaker), pp. 3–27. National Academy of Sciences, Washington, DC.
- Aschoff, J., E. Pöppel and R. Wever (1969) *Eur. J. Physiol.* **306**, 58–70.
- Aschoff, J. and R. Wever (1962) *Naturwissenschaften* **49**, 337–342.
- Aschoff, J. and R. Wever (1976) *Fed. Proc.* **35**, 2326–2332.
- Boulos, Z. and M. Terman (1981) *Neurosci. Biobehav. Rev.* In press.
- Bruce, V. G. (1960) In *Cold Spring Harbor Symp. Quant. Biol.* **25**, 29–47.
- Colin, J., J. Timbal, C. Boutelier, Y. Houdas and M. Siffre (1968) *J. Appl. Physiol.* **25**, 170–176.
- Cone, A. L. (1974) *Bull. Psychon. Soc.* **2**, 389–391.
- Czeisler, C. A. (1978) *Human Circadian Physiology: Internal Organization of Temperature, Sleep-Wake and Neuroendocrine Rhythms in an Environment Free of Time Cues*. Ph.D. Dissertation, Stanford University, Stanford, CA.
- Czeisler, C. A., G. S. Richardson, R. M. Coleman, J. C. Zimmerman, M. C. Moore-Ede, W. C. Dement and E. D. Weitzman (1981) *Sleep* **4**, 1–21.
- Czeisler, C. A., E. D. Weitzman, M. C. Moore-Ede, J. C. Zimmerman and R. S. Knauer (1980a) *Science* **210**, 1264–1267.
- Czeisler, C. A., J. C. Zimmerman, J. M. Ronda, M. C. Moore-Ede and E. D. Weitzman (1980b) *Sleep* **2**, 329–346.
- De Candolle, A. P. (1832) *Physiologie Végétale*, Vol. II, pp. 854–862. Béchét Jeune, Paris.
- Elliott, A. L., J. N. Mills and J. M. Waterhouse (1971) *J. Physiol. (London)* **212**, 30–31P.
- Enright, J. T. (1965) In *Circadian Clocks* (Edited by J. Aschoff), pp. 112–124. North-Holland, Amsterdam.
- Findley, J. D., B. M. Mialer and J. V. Brady (1963) Technical Report, Space Research Laboratory, University of Maryland, Submitted to the National Aeronautics and Space Administration. (taken from Aschoff, J. 1967. *Life Sci. and Space Research V.* (Edited by A. H. Brown and F. G. Favorite), pp. 159–173. North-Holland, Amsterdam.)
- Goetz, F. C., J. Bishop, F. Halberg, R. B. Sothorn, R. Brunning, B. Senske, B. Greenberg, D. Minors, P. Stoney, I. D. Smith, G. D. Rosen, D. Cressey, E. Haus and M. Apfelbaum (1976) *Experientia (Basel)* **32**, 1081–1084.
- Hoffman, R. A., P. G. George and W. F. Barrows (1969) *Am. J. Physiol.* **217**, 1487–1489.
- Klein, K. E., H. Hermann, P. Kulinski, and H. M. Wegman (1977) In *Vigilance: Theory, Operational Performance and Physiological Correlates*. (Edited by R. R. Mackie), pp. 117–132. Plenum Press, New York.
- Kokkoris, C. P., E. D. Weitzman, C. P. Pollak, A. J. Spielman, C. A. Czeisler and H. Bradlow (1978) *Sleep* **1**, 177–190.
- Kronauer, R. E., C. A. Czeisler, S. F. Pilato, M. C. Moore-Ede and E. D. Weitzman (1981) *Am. J. Physiol.* In press.
- Lydic, R., W. C. Schoene, C. A. Czeisler, M. C. Moore-Ede (1980) *Sleep* **2**, 355–361.
- Marimuthu, G. and M. K. Chandrashekar (1979) *Chronobiologia* **6**, 129.
- McNew, J. J., R. C. Burson, T. Hoshizaki and W. R. Adey (1972) *Aerosp. Med.* **43**, 155–161.
- Miles, L. E. M., D. M. Raynal and M. A. Wilson (1977) *Science* **198**, 421–423.
- Mills, J. N. (1964) *J. Physiol. (London)* **174**, 217–231.
- Mills, J. N., D. S. Minors and J. M. Waterhouse (1978) *J. Physiol. (London)* **285**, 455–470.
- Moore, R. Y. (1978) In *Frontiers in Neuroendocrinology*, Vol. 5 (Edited by W. F. Ganong and L. Martini), pp. 185–206. Raven Press, New York.
- Moore, R. Y. and D. C. Klein (1974) *Brain Res.* **71**, 17–33.
- Moore-Ede, M. C., D. A. Kass and J. A. Herd (1977) *Am. J. Physiol.* **232**, 31–37.
- Moore-Ede, M. C., R. Lydic, C. A. Czeisler, B. Tepper and C. A. Fuller (1980a) *Sleep Res.* **9**, 275.
- Moore-Ede, M. C., R. Lydic, C. A. Czeisler, C. A. Fuller and H. E. Albers (1980b) *Neurosci. Abstrs.* **6**, 708.
- Moore-Ede, M. C. and F. M. Sulzman (1977) *Physiologist* **20**, 17–25.
- Nafarrate, A. B. and L. E. M. Miles (1981) In *Proceedings of the XIV International Conference, International Society for Chronobiology*. In press.
- Pittendrigh, C. S. (1960) *Cold Spring Harbor Symp. Quant. Biol.* **25**, 159–184.
- Pöppel, E. (1968) *Eur. J. Physiol.* **299**, 364–370.
- Richter, C. P. (1922) *Comp. Psychol. Monogr.* **1**, 1–55.
- Rohles, F. H. and G. Osbaldiston (1969) *Bibl. Primatol.* **9**, 39–51.
- Rusak, B. and I. Zucker (1979) *Physiol. Rev.* **59**, 449–526.
- Saunders, D. S. (1977) *An Introduction to Biological Rhythms*. Blackie, Glasgow.
- Siffre, M. (1964) *Beyond Time*. (Translated and Edited by H. Briffault), pp. 1–128. McGraw-Hill, New York.
- Sulzman, F. M., C. A. Fuller and M. C. Moore-Ede (1977a) *Physiol. Behav.* **18**, 775–779.
- Sulzman, F. M., C. A. Fuller and M. C. Moore-Ede (1977b) *J. Appl. Physiol.* **43**, 795–800.
- Sulzman, F. M., C. A. Fuller and M. C. Moore-Ede (1978) *Am. J. Physiol.* **234**, 130–135.
- von Holst, E. (1939) *Ergeb. Physiol.* **42**, 228–306.
- Wahlström, G. (1965) In *Circadian Clocks* (Edited by J. Aschoff), pp. 324–328. North-Holland, Amsterdam.