DISTINGUISHED ADDRESS

Circadian Timing[a]

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INTRODUCTION

For any living system, two aspects of timing can be distinguished: Within the organism, a multiplicity of interacting processes has to be coordinated in time (as in space), and externally temporal adjustment of the organism's behavior to that of other organisms and to changing conditions in the environment is also needed. In many ways, internal as well as external timing implies the measurement of time, for example, by the decoding of impulse frequencies within the central nervous system. To serve such purposes, various mechanisms can be imagined that do not necessarily represent permanently running clocks. There are, however, four prototypes of such clocks that have evolved in adaptation to the four main periodicities in the environment: the tides, day and night, the lunar cycle, and the seasons. Each of these environmental cycles represents a temporal program that repeats itself in regular intervals and is hence predictable. By incorporating into its organization a copy of such a program, an organism is enabled to adjust its activities to the periodically changing conditions, and to be prepared in advance of ensuing tasks, that is, to measure external time by means of a biological clock.[1]

The characteristics of the four biological clocks resemble those of self-sustaining oscillations.[2] They persist in constant conditions with a frequency that slightly deviates from those of the environmental cycles that they mimic. This is indicated by the prefix "circa" used to designate circatidal, circadian, circalunar, and circannual clocks. To be useful as a time-measuring device, a circa-clock must run in synchrony with its environmental cycle. This is achieved through entraining signals from periodic factors in the environment, the zeitgebers.[3]

The most ubiquitous and of prime importance are the circadian clocks, which have been documented in all eukaryotic species from unicellular organisms up to man.[4–6] Circadian clocks not only enable the organism to occupy a "temporal niche" in the environment, but also provide internal temporal order by imposing rhythmic variations on all functions including psychomotor performance and mental activities such as time perception. A discussion of these interactions requires a brief introduction into the basic principles of circadian rhythmicity.

THE CIRCADIAN SYSTEM

The major characteristics of circadian rhythms and their entrainment by zeitgebers can readily be described on the basis of experiments performed with human subjects

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[a] This paper is dedicated to Professor Colin S. Pittendrigh on the occasion of his 65th birthday.

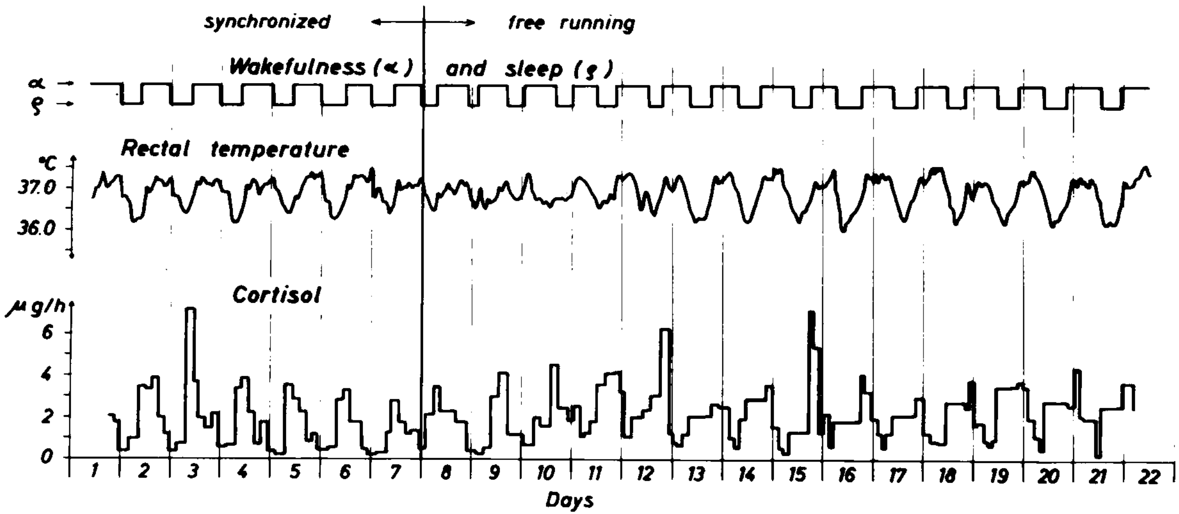

FIGURE 1. Circadian rhythms of wakefulness (α) and sleep (ρ), rectal temperature, and urinary cortisol excretion, recorded in a subject living alone in an isolation unit; for the first 7 days the subject was in contact with the experimenter (door open), but thereafter was without contact (door closed). (From Lund.[8] Reprinted by permission.)

under conditions in which external time cues are excluded or controlled by the experimenter. An underground isolation chamber has served such purpose in our institute since 1961.[7] It consists of a reasonably sized living room with bed, a small kitchen and a toilet with a shower. Subjects living in this unit for several weeks had to prepare their own meals and were usually asked to collect urine at short intervals as well as to perform a series of tests during the time they were awake. Their rectal temperature was recorded continuously, and they also indicated the times of waking up and of retiring by pressing buttons.

The results of a typical experiment are shown in FIGURE 1. For the first 7 days, the

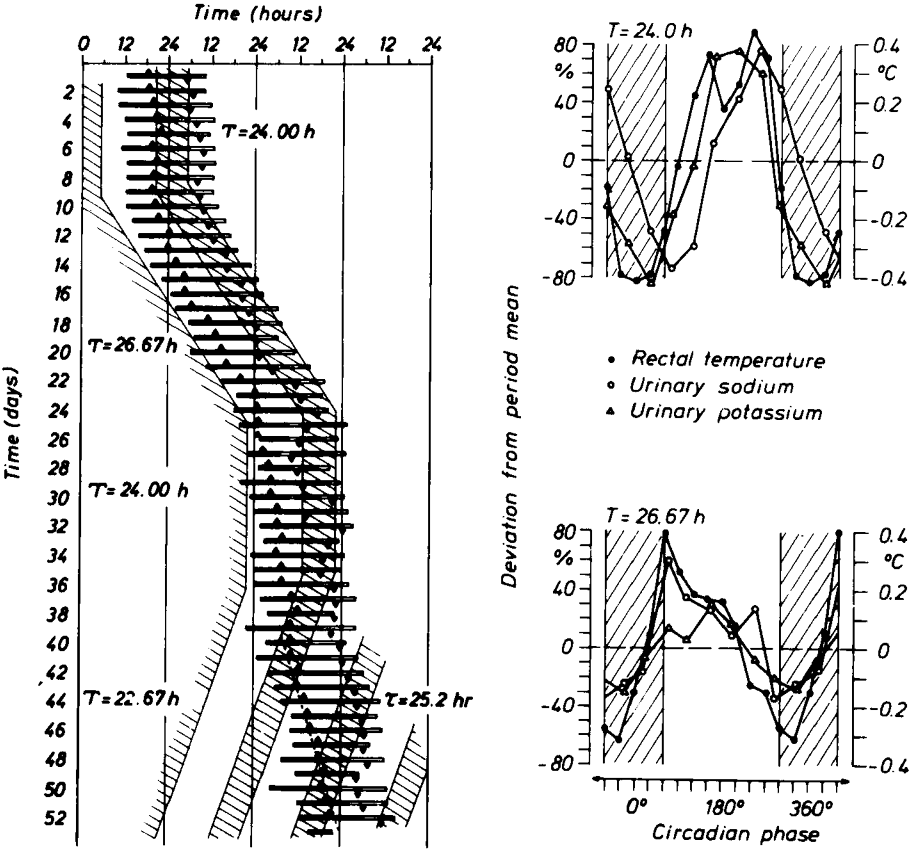

FIGURE 2. Circadian rhythms of a subject exposed in the isolation unit to an artificial zeitgeber with reading-lamp available. (*Left*) Black and white bars represent wakefulness and sleep, respectively; triangles = maxima (above the bars) and minima (below the bars) of rectal temperature; shaded area = darkness; T = zeitgeber period; τ = mean circadian period. (*Right*) Patterns of rhythms, averaged over several periods under T = 24 and T = 26.67 hr; shaded area = sleep. The abscissa is given in degree of a full circadian cycle, 360° representing 24 hr in the *upper* and 26.67 hr in the *lower* diagram. (After Aschoff *et al.*[10]; from Aschoff.[11] Reprinted by permission.)

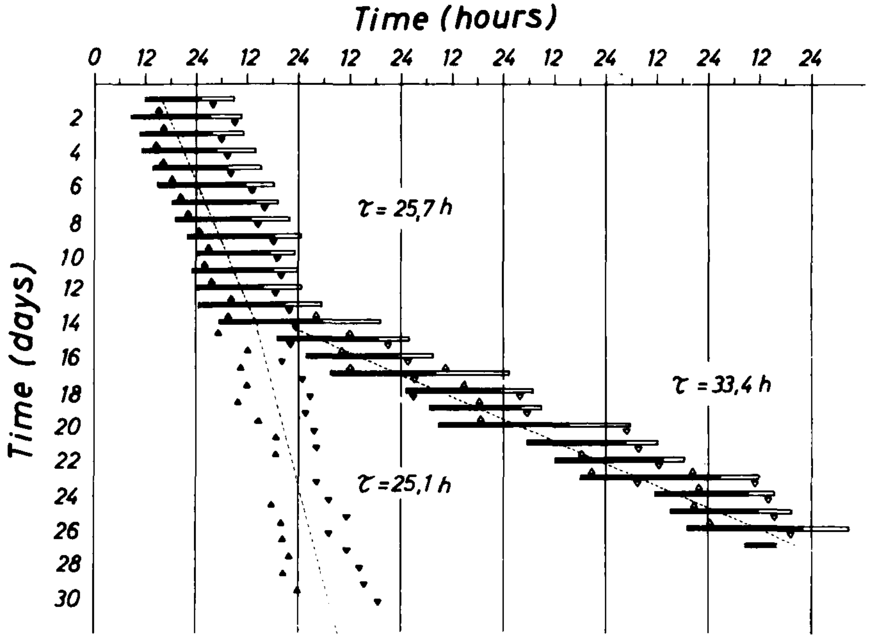

FIGURE 3. Circadian rhythms of wakefulness and sleep (black and white bars) and of rectal temperature (triangles above bars for maxima, below bars for minima) in a subject living alone in an isolation unit without time cues. Spontaneous internal desynchronization at day 16. τ = mean circadian period. (From Wever.[15] Reprinted by permission.)

door of the isolation unit was open, and the subject knew the time of day. Hence, the circadian clock was entrained to 24 hr, as shown by the rhythms in wakefulness (α) and sleep (ρ), in rectal temperature, and in the urinary excretion of cortisol. The subject was then isolated on the evening of day 7, and his rhythms were expressed as well as before, after a temporary initial disturbance. Closer inspection, however, reveals that the mean circadian period, measured for example, between successive awakenings or minima of rectal temperature, was no longer 24 hr, but lengthened to 26.1 hr, that is, the rhythm was free-running. In a sample of 147 subjects, the mean period (±S.D.) of free-running rhythms was found to be 25.0 ± 0.5 hr.[9]

In the isolation unit, human circadian rhythms can be entrained by light–dark cycles complemented by gong signals at regular intervals. Using this artificial zeitgeber, we have been able to synchronize subjects to periods other than 24 hr within certain narrow limits.[10] Noteworthy in these experiments is that the subjects had the choice of whether to follow the zeitgeber or not by making use of a small reading lamp. The example presented in FIGURE 2 shows an initial entrainment to a zeitgeber period T = 24 hr, thereafter to T = 26.67 hr, and finally failure of entrainment to T = 22.67 hr. In the 24-hr day, the subject was a "late riser," waking up several hours after the light was on (see the black and white bars for representations of wakefulness and sleep, respectively). In the longer day, he became, to his own surprise, an "early riser," eventually waking up even before the light was turned on. In the final part of the experiment, the subject did not follow the short period of the zeitgeber; his rhythm started to "free-run," with a mean period of 25.2 hr. When the subject changed from a late to an early riser, he also changed his internal temporal order. In the long day, the maxima and minima of rectal temperature (see the triangles in the left diagram of FIGURE 2) were advanced relative to the sleep-wake cycle. This difference in the internal phase relationship is more obvious in the two diagrams on the right of FIGURE 2, which shows the patterns of rectal temperature and of urinary excretion during the two conditions of entrainment: In the 24-hr day, the maxima of the rhythms occurred in late afternoon, in the long day shortly after waking. Similarly, the minima were shifted, and the wave form was drastically altered in its skewness.

The dependence of the external phase relationship between rhythms and zeitgeber, and of the internal phase relationship between rhythms and the sleep-wake cycle, on the period of the entraining zeitgeber is typical for any oscillation that is driven by another oscillation.[12,13] The relevance of this rule will be discussed again in the section on anticipatory activity.

In the experiments mentioned so far, all the rhythms recorded in one subject were free-running with the same frequency, that is, they were internally synchronized with each other (compare FIGURE 1, and the last part of the experiment shown in FIGURE 2). In contrast, free-running rhythms can occasionally split into two components which run with different frequencies.[14] An example of such "internal desynchronization" is given in FIGURE 3.[15] For the first 14 days, the rhythms of this subject were free-running with a common period of 25.7 hr. At day 15, the sleep-wake cycles spontaneously lengthened to a period of 33.4 hr, while the rhythm of rectal temperature continued to free-run with a period close to 25.0 hr. Spontaneous internal desynchronization has been observed in about 30% of all our subjects studied in constant conditions. These findings suggest that the circadian system consists of a multiplicity of oscillators, differentially controlling the sleep-wake cycle on the one hand and autonomic rhythms on the other. These oscillators are normally synchronized with each other, or kept in synchrony by the zeitgebers, but they can become uncoupled under certain, not very well understood, conditions. The phenomenon of internal desynchronization is of special interest with regard to time perception in isolation (see the subsequent section TIME PERCEPTION IN THE CIRCADIAN DOMAIN).

In the past 20 years, much has been learned about circadian clocks, their cellular and molecular mechanisms,[65] oscillatory characteristics,[66] and physiology.[6] There is ample evidence that the clock represents a multioscillatory system which comprises driving (self-sustaining) and driven (self-sustaining as well as damped) units. It is also clear that central pacemakers control the whole system. They are located in the hypothalamus, for example, in the nuclei suprachiasmatici (mammals), and in the pineal body (birds and lower vertebrates), but recent findings indicate that pacemaker-like structures may also exist elsewhere in the central nervous system (for discussion see Aschoff *et al.*[16]).

CIRCADIAN CONTROL OF PERFORMANCE

It has been known for a long time that the efficiency of performance varies with time of day. Prominent examples are provided by rhythms in reaction time, as demonstrated in FIGURE 4 for optical signals (four independent studies) and for acoustical signals (two studies). It has often been emphasized that these rhythms are more or less mirror images of the rhythm in rectal temperature, and a causal relationship between temperature and reaction time has been postulated.[23] Such conclusions are dangerous because they are based on correlations between processes that are programmed in time and may well be driven independently by coupled circadian pacemakers. Several sets of data contradict the hypothesis that reaction time is dependent on body temperature.[64] In one study, multiple-choice visual reaction time was measured in seven members of a crew during a sea voyage.[24] Tests were made at 4-hr intervals during 4 to 6 consecutive days in four different months. As shown in FIGURE 5, there was a strong negative correlation (r = −0.90 to −0.97) between reaction time and body temperature. There were also considerable changes in the mean levels of reaction times and body temperatures calculated on a 24-hr basis (see the dashed horizontal lines in FIGURE 5). These values were positively correlated with each other (r = 0.93). The changes in level made it possible to correlate measurements made in one subject in different months either at different times of day or at the same time of day, the latter approach excluding the time-of-day effects. As can be seen from FIGURE 6, the coefficients of correlation are all negative when data taken at different times of day are correlated (upper histogram), but become randomly distributed around zero when time-of-day effects are excluded (lower histogram). In the meantime, we have learned that it is the

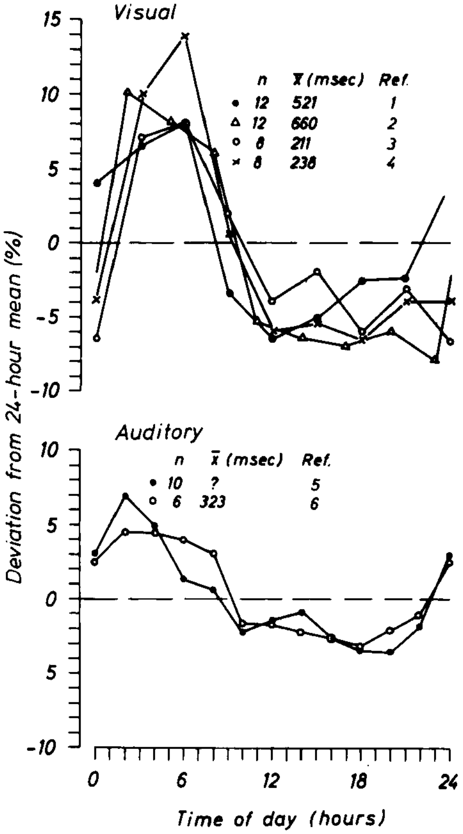

FIGURE 4. 24-hr variations in visual and auditory reaction time obtained in studies at seven different laboratories. n = number of subjects; $\bar{x}$ = 24-hr mean. Sources in *upper diagram*: ● = Ref. 17; △ = Ref. 18; O = Ref. 19; x = Ref. 20; ▲ = Ref. 21. Sources in *lower diagram*: ● = Ref. 22; O Ref. 8 ("blind" subjects).

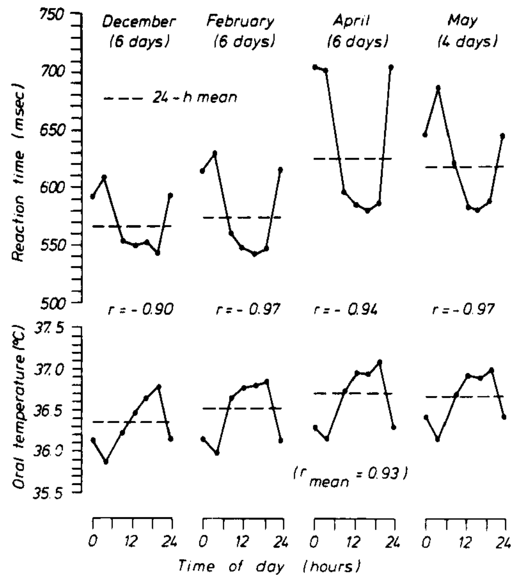

FIGURE 5. 24-hr variations in visual reaction time and oral temperature. Means of seven subjects, tested at four different months during a sea voyage. Each curve represents an average from measurements made during at least four consecutive days. (After Mann *et al.*[24])

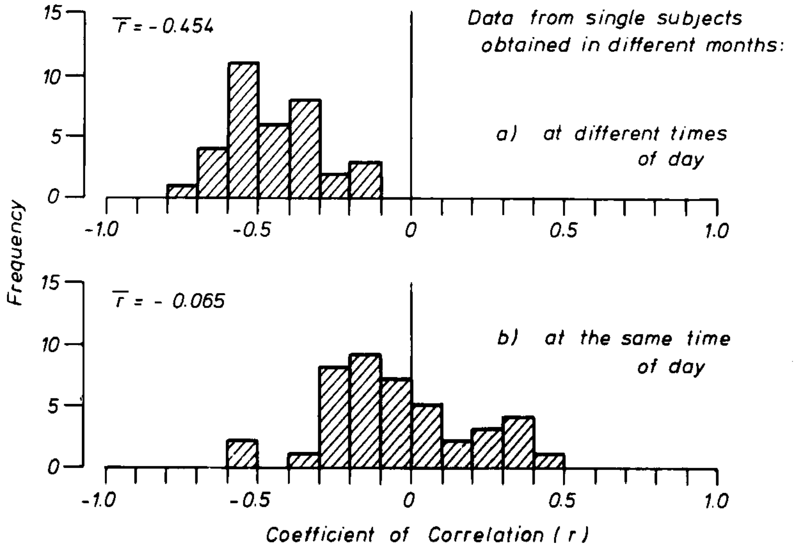

FIGURE 6. Coefficients of correlation between visual reaction time and oral temperature. Tests were made in 4-hr intervals during at least four consecutive days at four months of a sea voyage. (Data base as in FIGURE 5; after Mann *et al.*[24])

kind of task, and especially its memory load, which determines the phase relationship between the rhythms of performance and of body temperature.[25] Using a letter cancellation test, Folkhard and coworkers[26] have shown that the performance rhythm runs in phase with that of rectal temperature in a two-letter target, but counterphase in a six-letter target (FIGURE 7). These and other findings suggest that performance rhythms may be controlled by a variety of circadian oscillators.[27]

Even very simple motor performances can show strong circadian variations. The speed of tapping has been found to depend on circadian phase, both in tapping at the highest possible rate and in comfort tapping (that is, at a rate preferred by the subject).

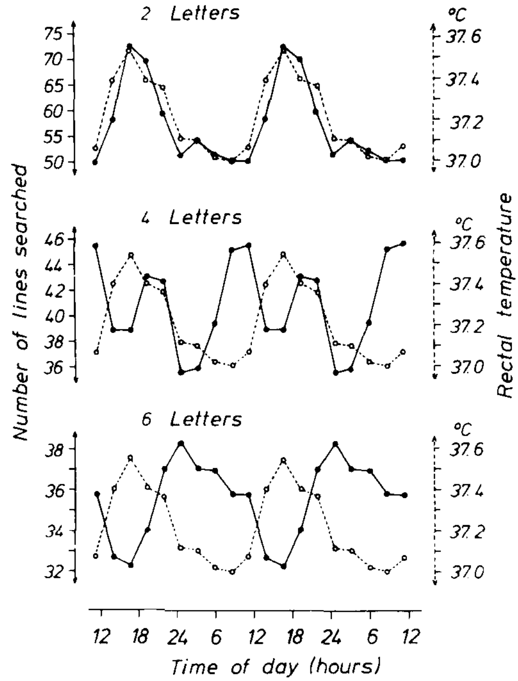

FIGURE 7. 24-hr variations in rectal temperature and in letter cancellation tests of different memory load (2-, 4-, or 6-letter target). Means from two subjects working for 18 days on a fast rotating shift schedule. (From Folkard.[25] Reprinted by permission.)

Interestingly, the data presented in FIGURE 8 indicate that females have a lower rate of fast tapping than do males, but prefer a higher speed in comfort tapping. The estimation of short time intervals also varies with time of day, as illustrated in FIGURE 9 for the production of 10-sec intervals and the reproduction of 6-sec intervals. Again, a correlation between the rhythms of time estimation and of body temperature should not be taken as evidence of a causal relationship, a hypothesis that has been considered to be supported by some findings,[30] but is contradicted by others.[31]

Most of these rhythms do not depend on the presence of a light–dark cycle. We have measured the estimation of short time intervals (production of 10 sec), together with various other performances and physiological functions, in six subjects who lived first for 4 days in a light–dark cycle (LD), and thereafter for 4 days in continuous darkness (DD), with a controlled sleep time from 23:15 to 7:30. No differences were found in either the amplitude or the phase of all rhythms between the two conditions.[32] The results of the 10-sec estimation task are reproduced in FIGURE 10. This time estimation was the only rhythm in which a slight difference was discovered: the 24-hr mean (±S.D.) was 10.84 ± 0.51 in LD, and 10.17 ± 0.46 in DD (difference significant to $p < 0.001$). I am not aware of any other study in which an effect of light or darkness on short time estimates has been demonstrated.

Furthermore, many performance rhythms persist during prolonged sleep deprivation.[17,33-37] Under those conditions, the range of oscillation (the "amplitude") may either increase, mainly due to a lowering of night values,[37] or decrease, depending on the kind of task.[36,38] An exception seems to be short time estimation, the rhythm of which usually disappears in subjects deprived of sleep. This is illustrated in the upper two diagrams of FIGURE 11; the lower two diagrams show the persistence of the tapping rhythm during sleep deprivation.

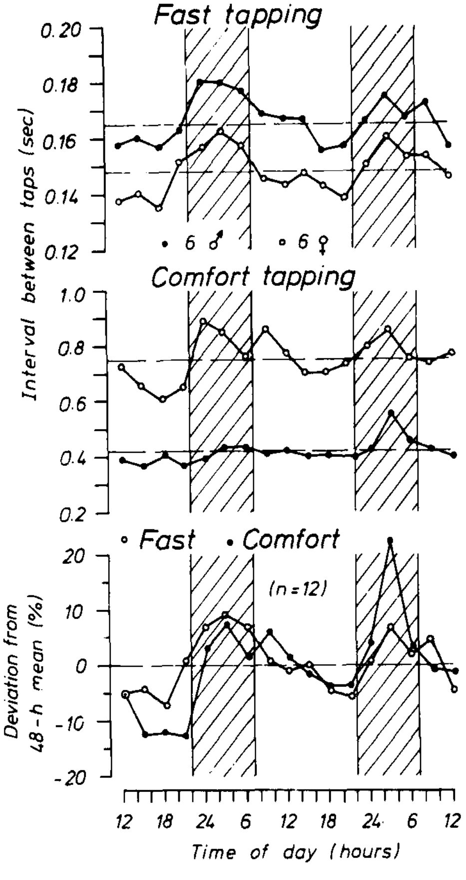

FIGURE 8. 24-hr variations in the speed of fast tapping (at the highest possible rate) and comfort tapping (at the preferred rate) measured in 3-hr intervals in six female and six male subjects during 48 hr with controlled sleep (*shaded area*). (From Winnewisser.[28] Reprinted by permission.)

Finally, circadian rhythms in performance have been demonstrated to free-run in

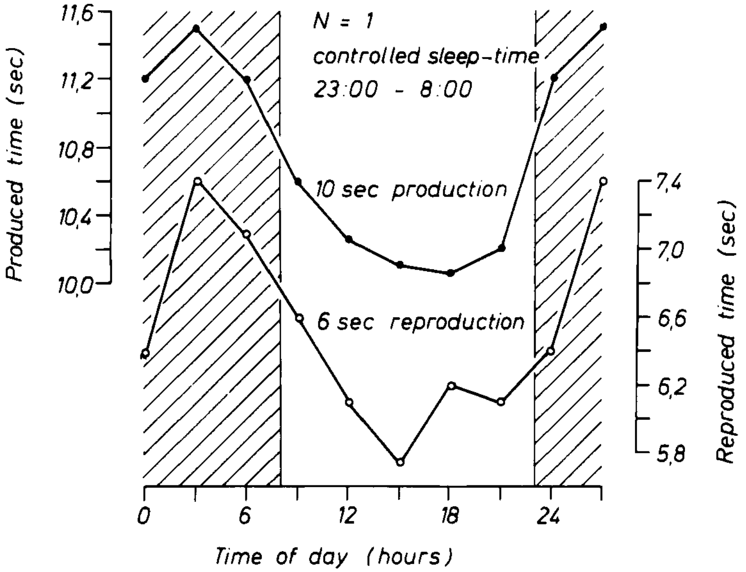

FIGURE 9. 24-hr variations in the production of 10-sec estimates, and the reproduction of 6-sec, measured in one subject during six days with a strict schedule of wakefulness and sleep (sleep time *shaded*). (From Pöppel and Giedke.[29] Reprinted by permission.)

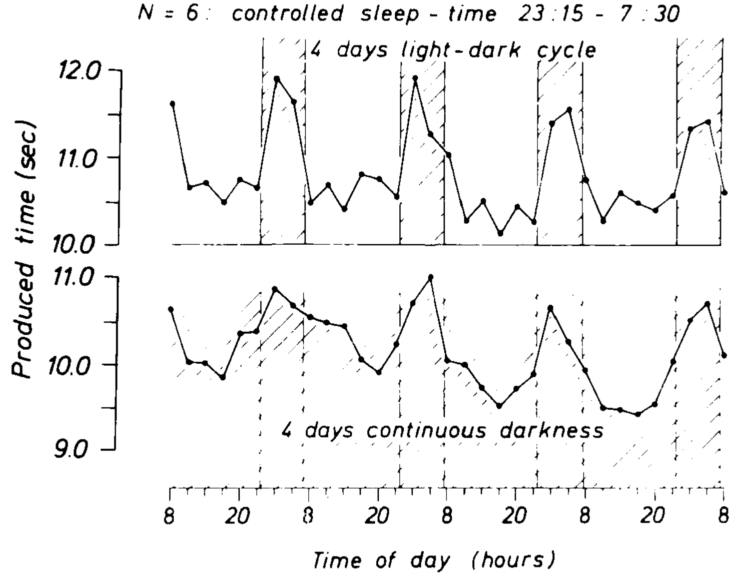

FIGURE 10. 24-hr variations in the production of 10-sec estimates. Means from six subjects who lived, first, for 4 days in a light–dark cycle, and thereafter for 4 days in continuous darkness. Sleep time under both conditions was from 23:15 to 7:30. Shaded area = darkness. (From Pöppel and Giedke.[29] Reprinted by permission.)

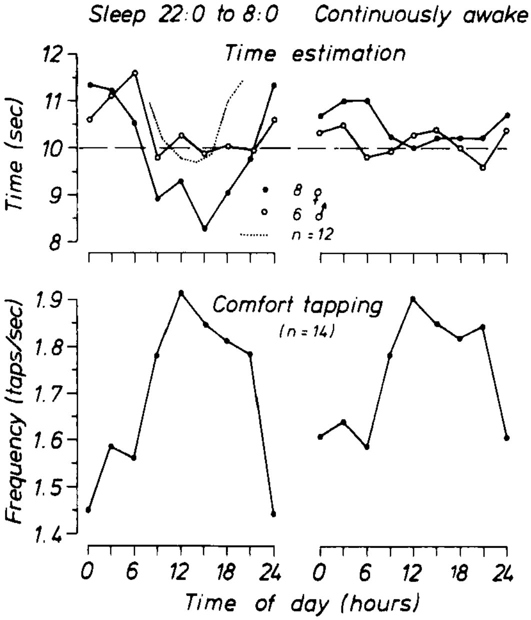

FIGURE 11. 24-hr variations in the production of 10-sec estimates and in the speed of comfort tapping, measured during a schedule with either sleep from 22:00 to 8:00 (*left*) or during sleep deprivation (*right*). Means of n subjects.

subjects living in isolation without time cues. A few examples are provided in FIGURE 12.[17] It should be pointed out, however, that the rhythm in time estimation shown in FIGURE 12 barely reached a level of statistical significance; in other isolation studies, no rhythm in the production of 10- or 20-sec estimates could be found (see the section TIME PERCEPTION IN THE CIRCADIAN DOMAIN). In cases of internal desynchronization, a performance rhythm may either follow the rhythm of rectal temperature or the sleep–wake-cycle. This has been shown in subjects who were exposed in the isolation unit to a "strong" zeitgeber, that is, a light–dark cycle (and gong signals) without the availability of a reading lamp.[27] Under those conditions, the subjects were forced to adhere to the zeitgeber with their sleep–wake cycles. The results of a typical experiment are reproduced in FIGURE 13. When the period of the zeitgeber was lengthened to 28 hr, the sleep–wake cycle remained entrained, but the rhythms of rectal temperature and of performance (computation task according to Pauli) started to free-run with a mean period of 24.8 hr ("forced internal desynchronization"). After a further lengthening of the zeitgeber period to 32 hr, rectal temperature still had a period of 24.8 hr, but the computation rhythm was now in synchrony with the sleep–wake cycle.

INTERACTIONS BETWEEN ULTRADIAN AND CIRCADIAN RHYTHMS

Many animals, especially small mammals, show a rhythmic alternation between activity and rest in short intervals of 1 to 3 hr in duration.[39] These ultradian rhythms are sometimes very precise, suggesting the existence of an ultradian pacemaker. The activity of a field vole (FIG. 14), recorded under natural photoperiodic conditions between March and June at a latitude of 47°N, illustrates the regularity at which bursts of activity occur, especially in early spring, when the animal is mainly day–active. Beginning in May, nocturnal activity predominates, a shift in phase that has been observed in a variety of rodent and fish species.[41,42] In the data of March and April, a few pecularities can be noticed: one burst of activity is strongly coupled to sunrise; the sequence of bursts becomes progressively less regular during the day; at night, the inter-burst interval (the ultradian period) is apparently longer than during the daytime.

In constant conditions, ultradian rhythms may persist with unchanged or even improved regularity. This is illustrated in FIGURE 15 by the actograms of three common voles *Microtus arvalis,* kept either in light–dark cycles (LD) or in continuous darkness (DD). In addition to the ultradian rhythms, all three animals show a circadian component (least expressed in animal 202). Several observations are noteworthy: (1) The ultradian rhythm is sometimes less pronounced in LD than in DD. (2) When free-running in DD, both components seem to remain more or less in synchrony with each other (animals 202 and 201), and a certain phase relationship is often kept between one of the ultradian bursts and the onset of the circadian activity component. (3) In a free-running rhythm, the onset of the circadian component can "jump" from one ultradian burst to a neighboring one (animal 205).

Actograms like those presented in FIGURE 14 and 15 pose several questions: (1) Is there a hierarchical order between the two components, that is, are the frequencies related to each other in integer units? (2) If the answer to (1) is negative, what effects

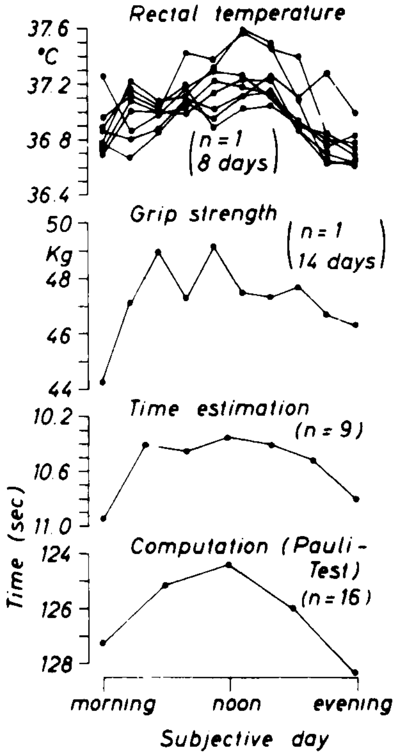

FIGURE 12. Circadian rhythms in rectal temperature, grip strength, production of 10-sec estimates and computation speed (Pauli test). Means of different subjects who lived for various time spans in an isolation unit without time cues. (From Aschoff *et al.*[17] Reprinted by permission.)

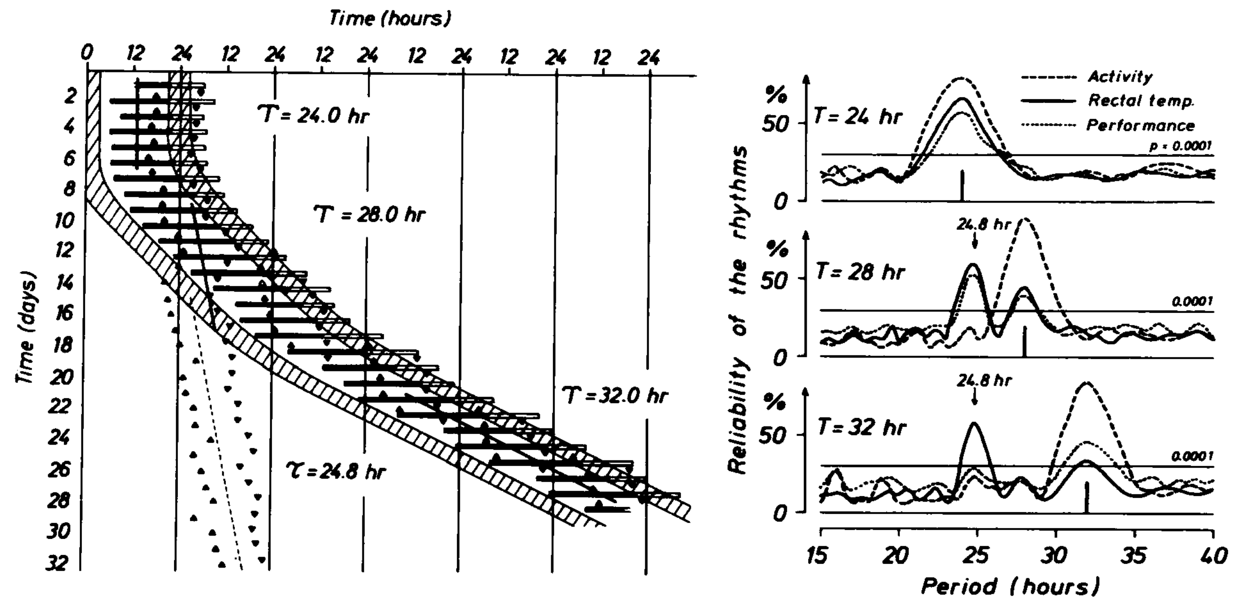

FIGURE 13. Circadian rhythms of a subject living in the isolation unit under the influence of an artificial strong zeitgeber (light–dark cycle with gong signals, no reading-lamp available). (*Left*) The rhythms of wakefulness and sleep (black and white bars), of rectal temperature (triangles for maxima and minima), and of computation speed (solid lines connect the daily phases of maximal performance speed). Shaded area = darkness. T = zeitgeber period. τ = mean circadian period. (*Right*) Period analyses of the time series computed separately for the three sections of the experiment with different zeitgeber periods. (From Wever.[27] Reprinted by permission.)

can be seen of the circadian on the ultradian rhythm? (3) Are there effects of the ultradian on the circadian rhythm?

In answer to question (1): From the analyses of many actograms, it must be concluded that an integer relationship between the two frequencies is a rare exception. In nearly all instances, ultradian periods are not a submultiple of the circadian period, and an interspecific comparison reveals that the ultradian period is positively correlated with body size,[43] while the circadian period is size-independent.

In answer to question (2): In most actograms one can see a burst of activity which is neatly coupled to the onset of the main activity component (the activity time, α). In a light–dark cycle this means that one of the bursts usually occurs either around dawn or around dusk, depending on the activity mode of the species. Furthermore, the ultradian frequency often varies with circadian time. We have analyzed this effect in more detail in the actogram of mice, *Mus musculus*, by measuring the duration of the ultradian periods, separately for the activity time α and for the rest time ρ. Data were taken from 15 animals kept in LD, and 18 animals kept in DD. As shown by the histograms in FIGURE 16, the ultradian period was consistently shorter in α than in ρ under both conditions.

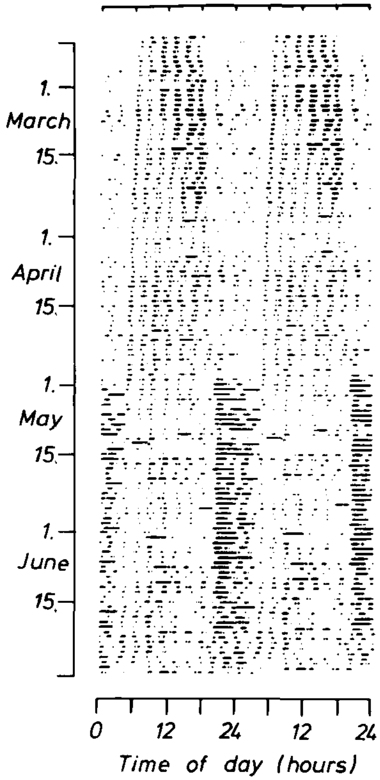

FIGURE 14. Activity pattern of a field vole, *Microtus agrestis*, recorded indoors under natural photoperiodic conditions in southern Germany. Original record plotted twice along the abscissa. (From Erkinaro.[40] Reprinted by permission.)

In answer to question (3): The "jumping" of the main activity onset from one ultradian burst to an adjacent burst, either to the preceding, as in FIGURE 15 (animal

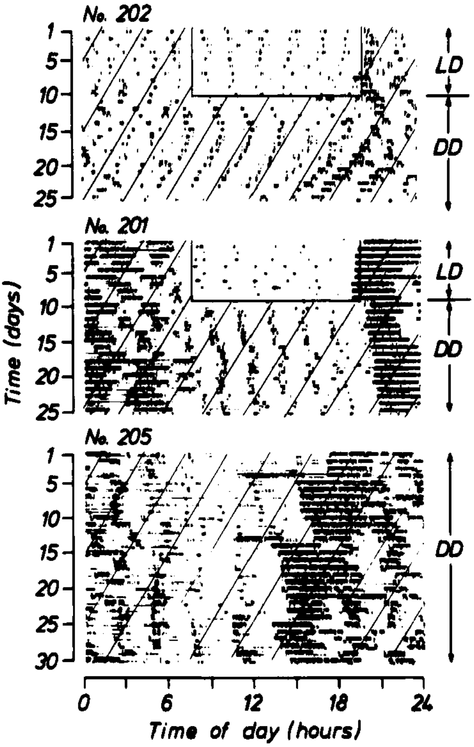

FIGURE 15. Activity patterns of three common voles, *Microtus arvalis*, recorded in the laboratory in either a light–dark cycle (LD) or in continuous darkness (DD). Courtesy of Menno Gerkema (unpublished data).

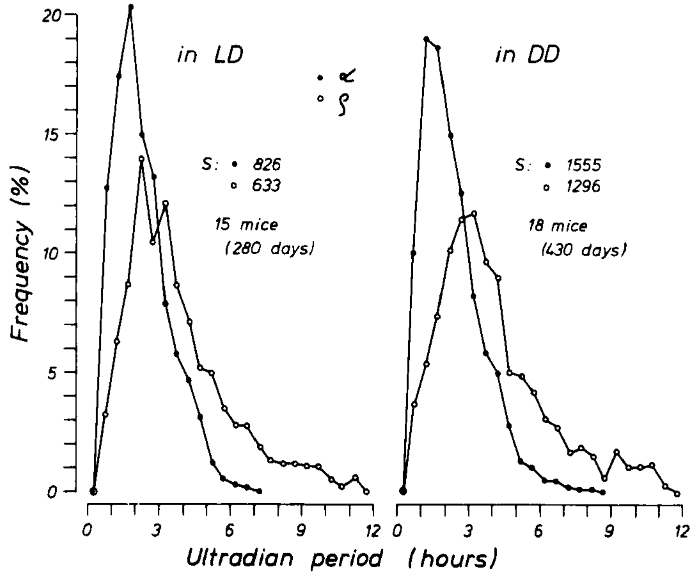

FIGURE 16. Frequency histograms of the duration of ultradian cycles in the activity of mice (*Mus musculus*) recorded in the laboratory either in a light–dark cycle (LD) or in continuous darkness (DD). The data were separately analyzed for the activity time α and the rest time ρ of the circadian rhythm. (Data from Aschoff and Meyer-Lohmann.[44])

205), or to the succeeding one, has been seen in only a few of the actograms recorded so far in constant conditions. The number of observations, however, justifies the hypothesis that there are effects of the ultradian on the circadian system.

In summary, the following conclusions can be drawn: Ultradian and circadian rhythms are usually not coupled in a strict hierarchical order; the circadian system can have a "phase-setting" effect on the ultradian rhythm and modulates its frequency; effects of the ultradian on the circadian system are suggested by the phenomenon of "jumping." It should be mentioned that all these interactions in one way or another also apply to the human circadian system and the REM/NREM cycle observed during sleep. The period of the REM/NREM cycle varies with circadian phase[45]; sleep-onset has a phase-setting effect on the first REM episode; the REM/NREM cycle is not a submultiple of the circadian period[46]; waking-up predominately occurs during REM[47,48], and in a similar way sleep onset or "sleepability" may be triggered by a certain phase of an ongoing ultradian rhythm, if there is one.[49]

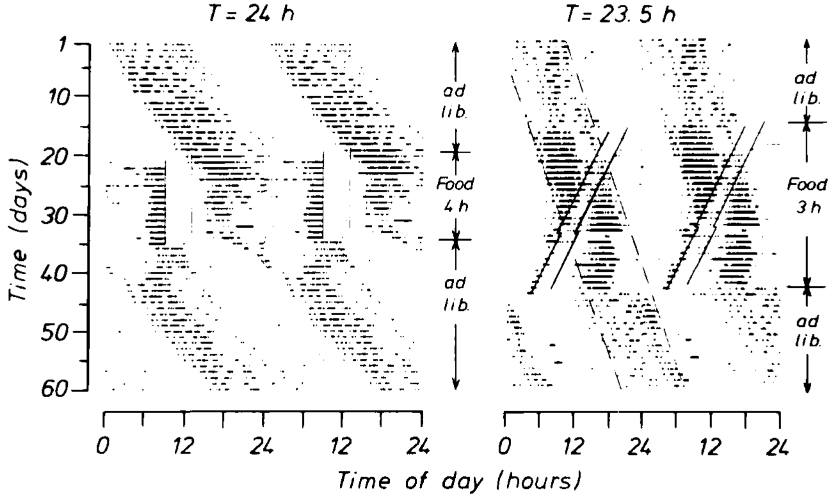

FIGURE 17. Activity rhythms of two rats kept in constant dim illumination and either fed *ad libitum* or for a few hours per day only. Meal times are indicated by two parallel *solid lines*. *Dashed lines* through onset and end of activity were drawn "by eye." T = interfeeding interval. Original records plotted twice along the abscissa. (From Aschoff *et al.*[55] Reprinted by permission.)

THE PROBLEM OF ANTICIPATORY ACTIVITY

A light–dark cycle represents the most powerful zeitgeber for the circadian rhythms of most organisms, but other environmental factors may also be effective, such as a cycle of high and low temperature[50] or of social signals.[51,52] It also has been postulated that circadian rhythms become entrained when food is offered periodically.[53,54] However, recent extensive studies have demonstrated that the activity rhythms of rats kept in otherwise constant conditions continue to free-run when *ad libitum* feeding is replaced by a schedule of offering food for a few hours per day only. The example provided in the left diagram of FIGURE 17 indicates that neither the phase nor the period of the free-running rhythm is altered by restricted feeding (RF), but it also shows a band of activity just prior to the time of feeding: the well-known anticipatory activity. Remarkably, this band of activity persists for several days after RF is again replaced by *ad libitum* feeding. From several such observations we have postulated that RF, although it does not act as a true zeitgeber, uncouples from the main circadian system a component of activity which has circadian-like characteristics.[56] If this assumption is correct, it could be expected that the duration of anticipatory activity depends on the period of the "entraining" feeding cycle (see the discussion in the previous section, THE CIRCADIAN SYSTEM, and FIGURE 2). To test this hypothesis, we have done a series of experiments in which the interfeeding interval, *T*, of RF was varied between 22 and 27 hr.[57] The results of an experiment with $T = 23.5$ (FIG. 17, right diagram) agree with the expectation as anticipation was shorter in $T = 23.5$ hr than in $T = 24$ hr. The summary of all results in FIGURE 18 demonstrates that the duration of anticipatory activity is positively correlated with *T* within the limits from 23.5 to 27 hr, and that no anticipation is left in $T = 22$ hr. Together with some other findings which cannot be discussed here in detail (see Aschoff *et al.*[57]), these findings support the notion that RF uncouples from the circadian system a subcomponent which has oscillatory capacities. The range of *T* values to which the subcomponent can be entrained seems to have narrow limits as is known from the circadian system.[13]

It has been reported that non-24-hr feeding cycles cannot be anticipated by rats which are entrained to 24 hr by a light–dark cycle.[58] This conclusion is based on experiments in which interfeeding intervals of 19 and 29 hr were used, that is, *T* values that probably were outside the range of entrainment for the subcomponent. We have repeated those experiments with *T* values closer to 24 hr in rats that were entrained to 24 hr by a light–dark cycle. Anticipation was seen in $T = 23.5$ as well as in $T = 25.0$ hr, and the duration of anticipatory activity depended on *T* in a similar way as in rats whose rhythms were free-running in constant darkness.

Anticipatory activity has often been characterized as the result of "learning," but I have not seen any explicit hypothesis on what mechanism such learning could be based. Somehow, it has to be a process of time measurement, but of what kind? The circadian system can hardly be used for this measurement, because rats anticipate feeding intervals that deviate from the period of free-running as well as of entrained circadian rhythms. The assumption of a circadian subcomponent that can be uncoupled from the main circadian system and is entrainable by restricted feeding is compatible with all observations made so far. Anticipatory time measurement cannot be based on the circadian pacemaker, as shown by the persistence of free-running rhythms during RF as well as by the observation that anticipation, with its typical dependence on *T*, occurs in animals that have been made arrhythmic by creating lesions in the nuclei suprachiasmatici.[59] It remains to be seen whether other pacemaker-like structures are involved.

TIME PERCEPTION IN THE CIRCADIAN DOMAIN

Subjects living in isolation units without external time cues soon become unaware of the real passage of time and even lose interest in it.[7] Experiments performed over the past 20 years with more than 250 volunteers have convinced us that only rarely can a subject judge with what circadian period he may have been living, and estimate, at the end of the experiment, how many real days he had been in the unit. Most subjects believe that they follow a more or less "normal" (that is, 24-hr) schedule of wakefulness and sleep despite the fact that the cycle usually exceeds 25 hr and can be much longer. A subject who developed a circa-bi-dian sleep–wake cycle with a period of about 50 hr estimated his stay in the unit half as long as it was in reality. In short, the passage of time is consistently underestimated.[7,60,63]

To learn more about time perception in conditions of isolation we have done several series of experiments in which the subjects were asked to press a button whenever they thought that 1 hr had passed. At each of these time points, the subjects also had to press another button for the duration of estimated 10 or 20 sec. All signals were recorded outside the isolation unit for the full duration of the experiment, which usually lasted for 3 to 4 weeks. The data were collected many years ago, but were never published in detail. Consecutive estimates of 1-hr intervals, recorded in three subjects during 5 days of isolation, are presented in FIGURE 19. With few exceptions, the estimates were considerably longer than 1 hr. They also showed large variations within a day, but the daily means remained fairly stable. From several of the curves reproduced in FIGURE 19 one further gets the impression that there were two maxima

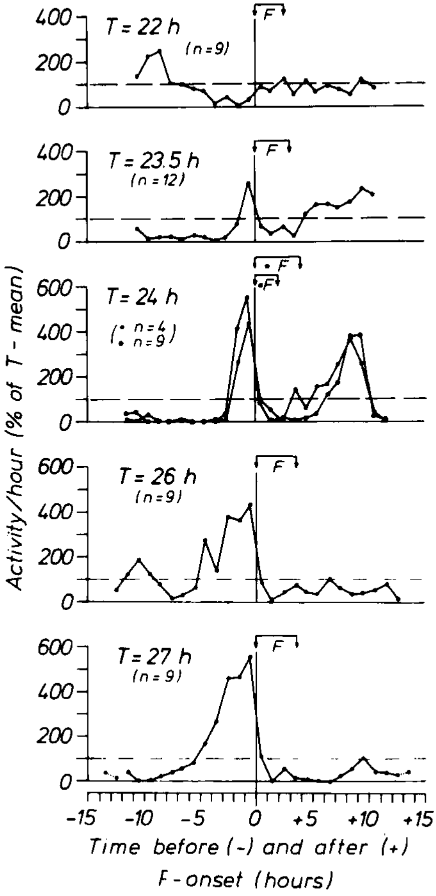

FIGURE 18. Activity patterns of rats kept in constant dim illumination and fed for a few hours per day only. F = feeding time, T = interfeeding interval. The hourly amount of activity is expressed in percent of the mean activity recorded in a full T cycle. n = number of animals. (From Aschoff *et al.*[57] Reprinted by permission.)

per day, one in the morning and another one in the afternoon. On the assumption that there was an underlying bimodal distribution of estimates, we attempted to average the data from many days and several subjects. To make this possible, despite interindividual differences in period length of the free-running rhythms, we normalized the duration of wakefulness to 100%, divided it into 10 segments of equal duration (= 10% of α), and assigned each 1-hr estimate to the segment in which it ended. In doing so, we could average the data from many days irrespective of the duration of α. Finally, the 10 values were expressed in percent deviations from α-mean. The resulting curves (FIGURE 20), obtained from seven subjects in 1961–62 and seven subjects in 1965–66, support the hypothesis that 1-hr estimates have a bimodal circadian distribution. An explanation for this is offered by the concept of "filled" and "empty" time or the "level of behavior."[61] The underestimation of the passage of time is greater when subjects are

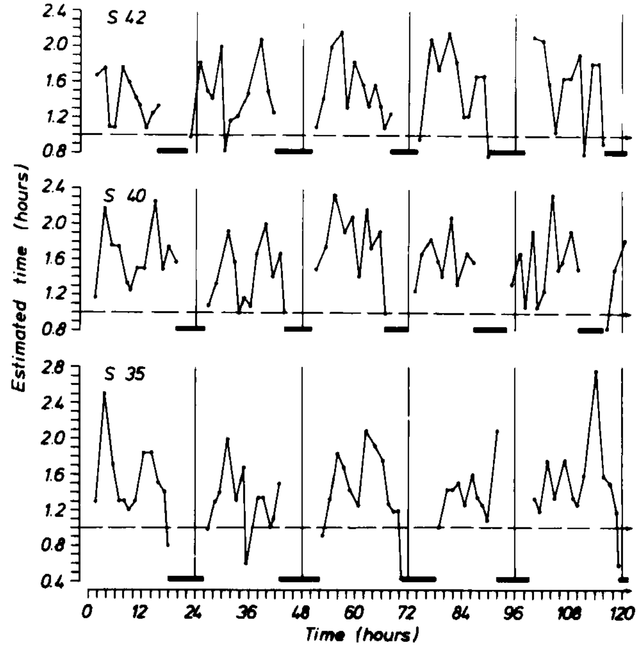

FIGURE 19. Consecutive estimates of 1 hr made by three subjects (S) who lived singly in an isolation unit without time cues. Horizontal bars = sleep.

alert and engaged in activities (late morning and afternoon), and the underestimation is smaller when subjects feel sleepy and bored, as is often the case during the "saddle" of efficiency around noon.

In a further step, we have calculated means of 1-hr estimates for every "day," and have related these values to the length of time the subject was awake during that day. As can be seen in FIGURE 21, there was a strong positive correlation between the production of 1-hr estimates and the duration of wakefulness, α, in all subjects. This is of special interest in cases of internal desynchronization where α was lengthened to 26 hr and more (subjects 5 and 7). In contrast to these very consistent findings in 1-hr estimates, the correlation between the estimation of 10 sec and α was only slightly positive in some subjects, negative in others, and also often close to zero. This is

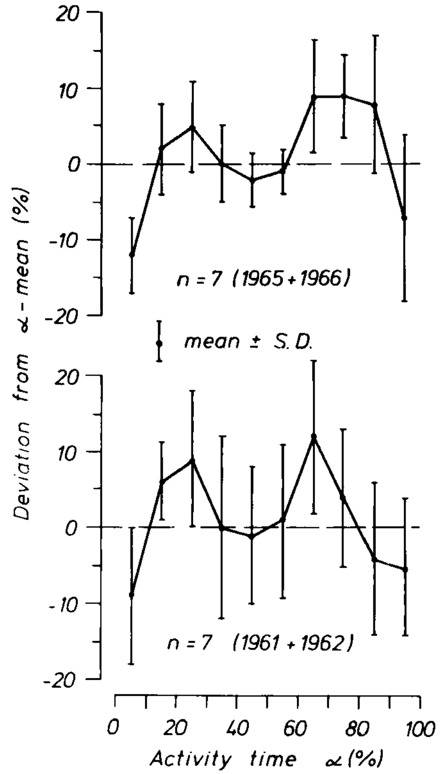

FIGURE 20. Circadian variations of 1-hr estimates. Means of two groups of seven subjects who lived singly in an isolation unit without time cues. For each single circadian period, the full time of wakefulness (activity time, α) was set as 100% and divided into ten classes of equal duration.

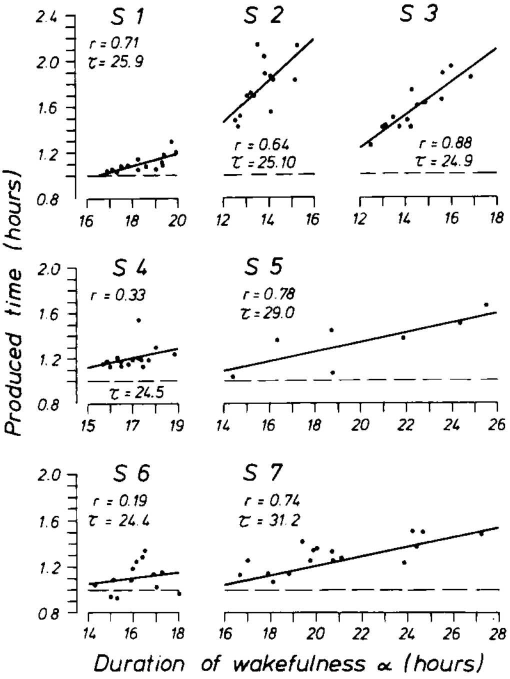

FIGURE 21. The dependence of 1-hr estimates on the duration of wakefulness, α, measured in seven subjects (S) who lived singly in an isolation unit without time cues. Each dot represents the mean of estimates made during one "day." r = coefficient of correlation; τ = mean circadian period as measured during the full experiment.

indicated in FIGURE 22 by the data from four subjects (upper row) who again showed a clear positive correlation between 1-hr estimates and α (lower row). From the same set of data, correlations were also calculated between 1-hr estimates and the circadian period τ; they were also positive, but less pronounced than those between the 1-hr estimates and α. The 10-sec estimates were not systematically correlated with τ. The frequency histograms of all coefficients of correlation, summarized in FIGURE 23, demonstrate again that, in contrast to the 1-hr estimates, the estimation of short time intervals is neither correlated with α nor with τ.

The intraindividual positive correlation between 1-hr estimates and α is also true interindividually. In the attempt to demonstrate this, we have taken into account individual differences in the mean time estimation by calculating the variation (the range) of the daily mean 1-hr estimates from the overall mean of each subject; these variations, expressed in percentage of the overall mean, were then related to the similarly calculated variations of α. According to FIGURE 24, the coefficient of correlation between the variation in 1-hr estimates and the variation of α, computed interindividually, was +0.575. We also calculated, separately for each subject, the extent to which the 1-hr estimates came close to being directly proportional to α, with 100% representing a 1:1-relationship. A frequency histogram of these factors of proportionality is provided in the inset of FIGURE 24.

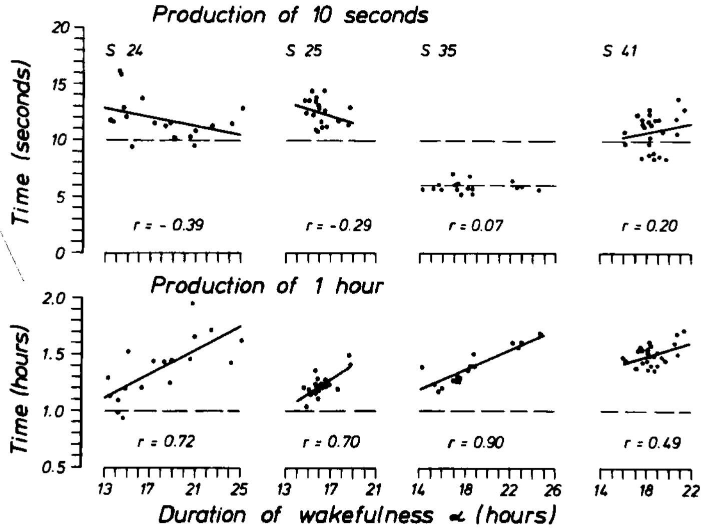

FIGURE 22. The dependence of 10-sec estimates (*above*) and 1-hr estimates (*below*) on the duration of wakefulness, α, measured simultaneously in four subjects (S) who lived singly in an isolation unit without time cues. Each dot represents the mean of estimates made during one "day." r = coefficient of correlation.

Two conclusions can be drawn from these findings: (1) The estimation of short time intervals (10 or 20 sec) seems to be based on a process radically different from that which is used to estimate longer time intervals such as 1 hr; hence, it is not too surprising that we could not find a dependence on circadian phase in the estimation of short time intervals, although it was well expressed in the 1-hr estimates (FIG. 20). (2)

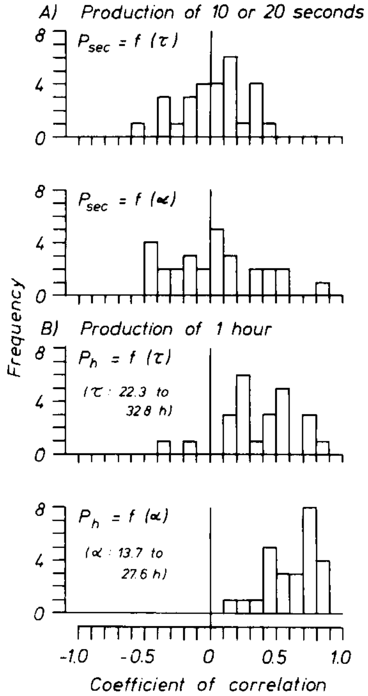

FIGURE 23. Frequency histograms of the coefficients of correlation between the production of short time estimates (P_{sec}) as well as the production of 1-hr estimates (P_h) and the duration of wakefulness (α) or the circadian period (τ), respectively. The interindividual variation of α and of ρ is given in brackets.

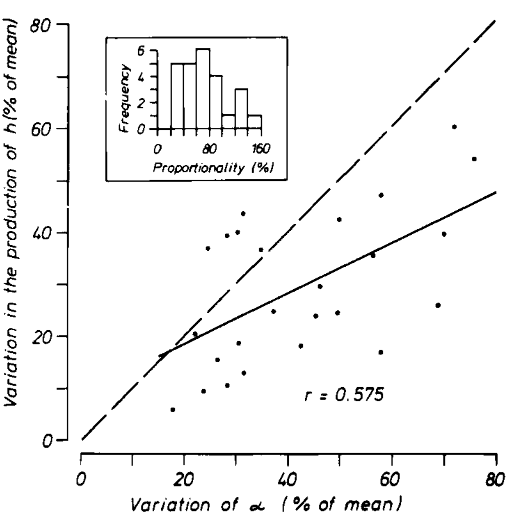

FIGURE 24. The variability of 1-hr estimates (expressed in percent of the mean of each subject) drawn as a function of the variation in wakefulness, α (expressed in percent of the mean α). Each dot represents the results from one subject who lived in the isolation unit without time cues. *Inset:* frequency histogram of factors representing the extent to which the variation in 1-hr estimates was proportional to the variation in α (100% = 1:1 proportionality).

The positive correlation between 1-hr estimates and α explains why subjects, despite their long and variable sleep–wake cycles and even during internal desynchronization, could believe that they were living on a normal 24-hr day because they produced about the same numbers of subjective "hours," irrespective of however long they were staying awake.

Intra- and interindividually, the 1-hr estimates vary less than the short time estimates. This is demonstrated in FIGURE 25 by frequency histograms which include all estimates made by six subjects (each line one subject) in the course of an experiment. The two insets of FIGURE 25 show the distribution of individual means for 10-sec estimates (18 subjects) and 1-hr estimates (25 subjects). To emphasize the difference in variability between short and long time estimation, consecutive daily means of time estimates are drawn in FIGURE 26 from 12 subjects who all made 1-hr estimates. In addition, four of these subjects were asked to produce 20-sec estimates (A), the other 8 to produce 10-sec estimates (B). The eight subjects included in (B) were arbitrarily divided into two groups of four each, according to individual differences in short time estimation. There were drastic differences in the overall means of 20-sec and 10-sec estimates (upper row of FIGURE 26). Some subjects were quite stable in their individual mean estimate, whereas others had a tendency to

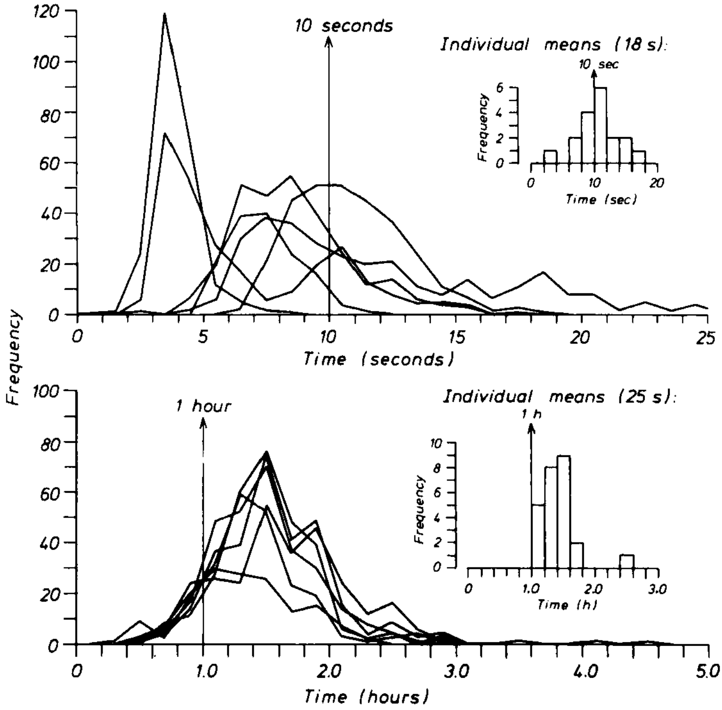

FIGURE 25. Frequency histograms of 10-sec estimates (*above*) and 1-hr estimates (*below*) measured in six subjects who lived singly in an isolation unit without time cues. Each curve includes all estimates made by a subject in the course of an experiment. *Insets:* frequency histograms of the individual means of 10-sec estimates (18 subjects) and of 1-hr estimates (25 subjects).

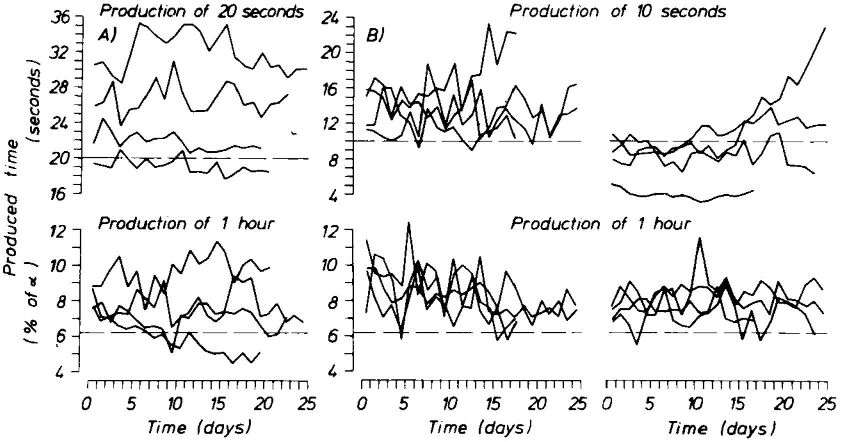

FIGURE 26. Consecutive daily means in the production of 20-sec or 10-sec estimates (*upper row*) and in the production of 1-hr estimates (*lower row*) measured simultaneously in subjects who lived singly in an isolation unit without time cues. (A) four subjects; (B) eight subjects, divided arbitrarily into two groups of four each according to individual differences in the production of 10-sec estimates.

lengthen the estimates in the course of the experiment. In the lower row of FIGURE 26, the 1-hr estimates are not given in real time but in percent of α. By this approach, the dependence of time estimation on α has been taken into account. (In a "normal" day with 16 hr of wakefulness, 1 hr amounts to 6.2% of α; see the dashed horizontal lines.) Most of the subjects kept to their individual mean throughout the experiment; in a minority an initial tendency to shorten was noticeable. All subjects except one produced means longer than 1 hr. The overall mean was about 1.4 hr, a value slightly longer than the 1.2 hr reported by Lavie and Webb[60] (mean of data from an exercising and a nonexercising group of subjects) and by Campbell[62] (data from nine subjects with 60 hr of bedrest). A more detailed analysis of time perception under conditions of prolonged temporal isolation will be published elsewhere.[68]

CONCLUDING REMARKS

There seems to be almost no process of timing and time perception that is not affected by the circadian clock in one or another way. Often, these influences result in a mere modulation of rate in, for example, the processes that underlie short-term performance. On the other hand, rhythmically timed events, such as the ultradian cycles in behavior, can be coupled to the circadian clock with unidirectional effects on the frequency of the ultradian system and bidirectional effects on the phases of both systems. Finally, of major importance, but presently least understood, are the effects of the circadian clock on the estimation of time in the range of hours. In this context, it is noteworthy that the timing of meals by subjects living in the isolation unit is correlated with the duration of wakefulness in a way similar to that observed in the 1-hr estimates. To give an example: the interval between waking up and lunch is more or less proportional to the time the subject stays awake.[67] These recent findings suggest that, during internal desynchronization, the extreme lengthening (or shortening) of the "circadian" period involves not only the sleep–wake cycle, but other physiological processes as well, possibly including metabolic rate. Under those aspects, new questions come up concerning the relationship between timing as well as time perception and the various components of the circadian system.

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