

Circadian Rhythms in Mammals: Formal Properties and Environmental Influences

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Abstract

Circadian rhythms are ~24 h cycles of behavior and physiology that are generated by endogenous biological clocks (pacemakers or oscillators). Circadian rhythms persist (“free-run”) with near 24 h periods (cycle lengths) in time-free environments but normally are synchronized to the 24 h day by environmental stimuli (zeitgebers or ‘time cues’). Synchrony is achieved by a process of entrainment, involving daily, stimulus-induced phase shifts that correct for the difference between the intrinsic period of the pacemaker and the period of the environmental cycle. Light is the dominant zeitgeber for most species and can induce phase shifts that vary in magnitude and direction depending on the circadian phase of exposure.

Circadian rhythms in some species can also be shifted and entrained by stimuli other than light, such as scheduled feeding, exercise, social interactions and temperature variations. Entrainment to feeding schedules is mediated by a circadian pacemaker that is separate from the pacemaker mediating synchrony to light-dark cycles. Interactions between photic and nonphotic zeitgebers are complex; e.g., light and exercise, depending on their relative timing and magnitude, may be synergistic or mutually inhibitory in producing phase shifts. Stable entrainment in natural environments therefore likely reflects integration of multiple zeitgebers by multiple, formally distinct pacemakers and oscillators.

Overt rhythms in behavior and physiology may emerge only weeks or months postnatally, but the circadian pacemaker responsible for entrainment to lighting cycles in adulthood is also entrained prenatally by maternal cues. Over the lifespan, circadian rhythms exhibit changes in period, amplitude and responsiveness to zeitgebers. Some of these changes may lead to altered circadian organization in older people, which may contribute to disruptions in physiological mechanisms, including those regulating sleep.

I. The Nature of Circadian Rhythms

A salient characteristic of sleep in humans is its daily rhythmicity. A similar 24 h rhythmicity characterizes the behavior, physiology and biochemistry of most living organisms. Historically, daily rhythms were interpreted as innate or acquired responses to environmental stimuli, such as light, temperature and humidity, that vary markedly with time of day. However, as early as 1729, it was reported that the daily rhythm of leaf movements of the heliotrope *Mimosa* does not depend on exposure to the day-night cycle, i.e., the rhythm persists ('free-runs') in constant dark (DD). Many studies have since confirmed that daily rhythms in a great variety of species, including humans, free-run, often indefinitely and with impressive precision, despite housing in controlled environments that lack variations in lighting, temperature, food access or other stimuli (e.g., Figs. 1, 3, 4) (1).

Persistence of daily rhythms for many cycles in the apparent absence of environmental time cues suggests that organisms possess one or more endogenous clock-like timekeeping mechanisms. If these mechanisms were biochemical processes within organisms, they might reasonably be expected to show dependence of rate (= frequency, the reciprocal of which is cycle duration or period, represented by the Greek letter τ) on tissue temperature, since chemical processes are almost universally temperature-dependent. Yet, for these mechanisms to time behavior and physiology accurately, they must be capable of cycling at a constant rate, despite variations in tissue temperature caused by changes in metabolism (for homeothermic species) or environmental conditions (for heterothermic species). The evidence accumulated in the 1950s that daily rhythms in most organisms show an impressive stability of period across a range of temperatures fueled a debate over whether daily rhythms were truly endogenously generated, or whether they reflected a response of the organism to some unknown, periodic environmental 'Factor X' associated with the earth's rotation on its axis (e.g., electromagnetic fields; 2).

Although the mechanism by which daily rhythms maintain a stable period at different temperatures remains to be fully elucidated, the evidence is now overwhelming that daily clocks are endogenously generated. The most compelling behavioral evidence in support of the endogenous clock concept is the observation that daily rhythms in constant conditions typically express stable τ 's that deviate from the 24 h periodicity of the solar day, and thus would rapidly slide out of phase with any geophysical correlate of planetary rotation. This 'circadian' (L. 'approximately a day') periodicity exhibits individual differences around a species-typical mean, usually within the range of 23–25 h. Consequently, individual animals housed separately but under identical environmental conditions gradually drift out of synchrony with local time and with each other. Moreover, a short (< 24 h) or long (> 24 h) circadian τ (reflecting a fast or slow running circadian clock, respectively) can be selected by breeding, induced by gene mutations (see Chapter xx), drugs, hormones or certain environmental manipulations, and may accompany aging (discussed further below). All of these observations are consistent with the hypothesis that

there is a genetically specified internal circadian clock that drives daily rhythms in behavior and physiology. Indeed, circadian rhythms are known to persist in constant conditions on space shuttles in orbit, completely removed from any hypothetical terrestrial Factor X (3).

Over the past few decades, circadian clocks have been localized in the brains and other tissues of several species, and genes and proteins that comprise the gears of these clocks have now been identified in cyanobacteria, a fungus, insects and mammals (for references, see chapter xx). Thus, the focus of research in this area is no longer on whether circadian rhythms are generated by internal clocks, but rather, what the components and processes of these clocks are, how they control behavior and physiology, how are they synchronized to the environment, and what impact they have on a variety of medical conditions. In addition, a major current focus is on understanding how our internal clocks might be manipulated to treat problems related to circadian timing, such as the disturbances of sleep, arousal and other physiological processes associated with shift work, rapid transmeridian jet travel, blindness, and other conditions. An understanding of the functional properties of circadian clocks is essential to set the framework for the cellular and molecular analyses that address these important questions. This chapter reviews the basic functional properties of circadian clocks, while subsequent chapters review the neurobiological and molecular genetic analyses of these clocks.

II. Parameters and measurement of circadian rhythms

Overt circadian rhythms have phase and amplitude dimensions (Fig. 2). Phase (conventionally represented by the Greek letter ϕ) refers to any point within a cycle. An observable phase (e.g., daily wake-up time) can be used as the hand of a clock to track its motion. The elapsed time between successive occurrences of a particular phase represents τ . The ϕ and τ of an observed circadian rhythm reflect parameters of the underlying circadian clock; namely, its current position and its rate of cycling, respectively. Amplitude refers to the range of values that an overt circadian rhythm can assume through the course of its cycle. One measure of amplitude is the difference between the peak value and the mean value of a purely sinusoidal circadian rhythm (the acrophase of a fitted cosine wave; e.g., Fig. 2). However, the amplitude of overt rhythms cannot be taken to represent a parameter of the underlying clock, because many factors downstream from the clock can affect the level and range of particular observable behavioral or physiological variables.

The experimental analysis of circadian rhythms has relied heavily on a few organisms that exhibit precise and easily measured circadian rhythms. The most popular model systems, and the favored rhythm for measurement, have included the bread mold *Neurospora* (daily rhythm of spore production), the fruit fly *Drosophila* (daily rhythm of activity or, for population studies, eclosion), the marine mollusc *Bulla* (compound action potentials recorded from the optic nerve) and several nocturnal rodents, particularly the Syrian hamster (daily rhythm of wheel running), the house mouse (wheel running) and the Norway rat

(wheel running, body temperature and sleep). Behavioral and physiological data are typically acquired by sensors whose outputs are monitored continuously by computers. Data stored in digital format are amenable to time-series analyses (e.g., linear regression, spectral analysis) using a variety of established techniques to measure ϕ and τ , and curve fitting procedures (e.g., cosinor analysis) to measure ϕ and amplitude (4,5,6). Despite the broad range of species studied and variables monitored, the formal properties of circadian rhythms are remarkably general, a feature consistent with an ancient origin for circadian clocks. In this chapter, we will refer primarily to the literature from studies of mammalian circadian rhythms.

III. Environmental Influences

A. Photic Entrainment

Circadian clocks are thought to have several important functions that enhance reproductive fitness in natural environments. A primary function is to promote consolidated periods of sleep (or rest) and wakefulness (and activity), and to restrict these to appropriate phases of the solar day, resulting in relatively stable 'nocturnal' (night active), 'diurnal' (day-active) or 'crepuscular' (dawn and dusk active) chronotypes that are consistent with the sensory and other adaptations of a given species. A fundamental advantage of using an internal clock to regulate rest-activity cycles is that physiological changes can be mobilized in anticipation of the transitions between day to night that herald the dramatic ecological differences between these daily phases. This adaptation allows for the expression of an anticipatory, rather than reactive, regulation of physiology, thus ensuring that the organism is fully prepared for activity or rest at the correct time. Humans and other diurnal species thus exhibit a gradual increase in body temperature, plasma cortisol and sympathetic autonomic tone beginning several hours before habitual wake onset, thereby facilitating a rapid switch from sleep to alert waking (7). Similar preparatory processes occur in reverse to facilitate sleep onset (e.g., body temperature declines, melatonin secretion begins).

In at least some species, the circadian clock has been exploited not only as a device for driving daily rhythms (i.e., a pacemaker function), but also as a true clock that can be continuously consulted to provide a sense of time. Evidence for this function is available from studies showing that some animals can discriminate time of day without external cueing, and thereby learn to associate the time and place of food availability (8,9). Some species use this time sense to navigate using celestial cues as a compass, which requires continuous adjustment of the angle of movement relative to the sun's apparent position in the sky (10). The circadian clock is also used by many species to measure daylength for the purposes of regulating reproduction, hibernation and other seasonally varying functions (11).

To achieve these adaptive functions, it is imperative that the circadian clock maintain an appropriate phase relation to local environmental time. This challenge is complicated by seasonal variations in daylength; outside the

equatorial zones, the hours of dawn and dusk shift systematically each day as the earth moves in its orbit around the sun. The circadian clock therefore has two tasks: it must generate a cycle to approximately match the period of the day–night cycle, and it must track a particular phase of the day–night cycle, so that diurnal animals become active near dawn, and nocturnal animals near dusk. The process by which this optimal synchronization occurs is called *entrainment*, defined as phase and period control of one oscillating process by another. In the case of the daily sleep–wake cycle and other circadian rhythms, entrainment to local environmental time is mediated by the actions of periodic environmental stimuli (so-called *zeitgebers*, German for 'time-givers') on the circadian clock.

As might be expected, light is a virtually universal zeitgeber for the circadian rhythms displayed by most organisms, from cyanobacteria to mammals. The mechanism by which light entrains circadian rhythms has been investigated by assessing the effects of discrete applications of light on circadian rhythms free-running in DD (12). Brief light pulses cause phase shifts of free-running rhythms, the magnitude and direction of which depend on when within the circadian cycle the light pulse is presented (Fig. 3a,b). This phase dependence of light effects is illustrated graphically by plotting the size and direction of the phase shift against the circadian phase of light presentation, producing a *phase–response curve* (PRC) for light (Fig. 3c).

The PRC for light pulses is universal in its general shape. Light exposure at the beginning of the 'subjective night' (when nocturnal species are normally active and diurnal species are asleep), induces a 'phase delay' shift, whereby the onset of the next 'subjective day' (rest phase in nocturnal species, active phase in diurnal species) is reset to a later time, and the circadian cycle resumes its free-run from this new phase. Light exposure toward the end of the subjective night induces a shift in the opposite direction, a 'phase advance', whereby the next subjective day begins earlier than usual. During most of the subjective day, light pulses have relatively little effect, defining a so-called 'dead zone' on the PRC.

The shape of the PRC for light pulses suggests that entrainment can be accomplished by a phase delaying action of light at dusk and a phase advancing action at dawn, producing a net daily shift equal to the difference between τ of the circadian pacemaker and the period (T) of the LD cycle (24 h in natural environments). Consistent with this hypothesis, known as the 'discrete' (or non-parametric) model of entrainment (13), organisms can be entrained to photoperiods consisting of only two brief light pulses in each 24 h cycle, simulating dawn and dusk (e.g., Fig. 3f). Stable entrainment under such "skeleton" photoperiods has been documented in several species and appears to be similar in most respects to entrainment under a full photoperiod. In fact, studies of semi-fossorial nocturnal rodents in seminatural environments indicate that self-selected brief exposures to light at dawn and/or dusk may account fully for entrainment in these species (14).

The τ of a circadian pacemaker and its circadian rhythm of sensitivity to light (summarized quantitatively in the PRC) determine several important

properties of entrainment. One property is the limited range of entrainment. The circadian clock can entrain to LD cycles only if there is a relatively close match between τ and T of the LD cycle. For stable entrainment to occur, the difference between τ and T generally cannot exceed the maximum daily phase shift that can be induced by exposure to available light. Thus, entrainment of humans to the solar day on Mars (24.6 h) is feasible (assuming a τ of 24.2 h, this requires a 24 min delay each day), whereas stable entrainment to the 18 h duty cycle on an American submarine crew should be virtually impossible (a 6 h advance being required each circadian cycle; 15).

In practice, the circadian range of entrainment can be extended if T of the LD cycle is changed gradually. This observation appears to be related to a partial modification of the intrinsic circadian τ towards the value of T . Thus, τ in DD tends to be longer following entrainment to $T > 24$ h, and shorter following entrainment to $T < 24$ h (16). These so-called ‘aftereffects’ on τ can persist for many weeks and suggest that entrainment limits are somewhat flexible because the intrinsic τ of the circadian clock can be modified by experience. Thus, for a given species there is a range of endogenous circadian periodicities that can be expressed, and observed τ will depend on both the current and recent environmental conditions.

A second property of entrainment predictable from τ , T and PRC shape is the phase relation between rhythm and zeitgeber during stable entrainment. The phase relation, or phase difference (abbreviated Ψ), is the difference in minutes or hours (or radial degrees, if expressed as a phase angle) between a definable circadian rhythm phase (e.g., wake-onset) and a definable zeitgeber phase (e.g., sunrise, in which case the phase relation can be abbreviated Ψ_{LD}). The case can be made that Ψ_{LD} is the ultimate target of natural selection for the value of τ and the shape of the PRC, a premise summarized indirectly in the aphorism that it is the early bird that gets the worm.

How might a bird enhance its chances of getting the pick of the breakfast buffet? One strategy is to have a short τ . Recall that stable entrainment will occur at whatever Ψ_{LD} results in the photic stimulation that generates a net phase shift that precisely compensates for the difference between τ and T . If $\tau < 24$ h, then a net phase delay is required each circadian cycle. A larger net delay will occur when evening light stimulates more of the phase-delay portion of the PRC, and when morning light stimulates less of the phase-advance portion. This will occur if the PRC is shifted slightly to the left (i.e., advanced) relative to environmental time. Thus, a bird with a short circadian τ will assume a more advanced (positive) Ψ_{LD} , resulting in an earlier onset of activity at dawn. By contrast, if $\tau > 24$ h, a net daily advance is required, stable entrainment will occur at a more negative Ψ_{LD} , and wake-up time will occur later relative to dawn. Within limits set by other factors (e.g., availability of light and prey for feeding), a short τ could confer an ‘early-bird’ feeding advantage.

Individual differences in τ may also account for the ‘early-bird’ and ‘night-owl’ phenotypes that we recognize among humans. In fact, an extreme form of

the early-bird phenotype (advanced sleep-phase syndrome) has been traced to a molecular change that affects the periodicity of the human circadian clock (17). Note that differences in Ψ_{LD} can also occur as a result of small differences in PRC shape or in the strength of the zeitgeber (e.g., the duration, intensity or wavelength of light exposure each day).

A third important property of entrainment predictable from τ and the PRC is the response of circadian rhythms to acute displacements of the LD cycle. If the LD cycle is advanced by 6 h, simulating a rapid trip from North America to Europe, the circadian clock re-entrains gradually, by small phase shifts (so-called "transient" cycles) over several days. The size and direction of these shifts is determined by τ , the intensity, wavelength and precise timing of light, and the shape of the PRC (specifically, the area under the delay and advance portions and the phase at which resetting crosses-over from delays to advances). Syrian hamsters, a favorite model species in chronobiology, have a species-typical τ very close to 24 h and a relatively large advance to delay ratio of their PRC (Fig. 3c). Not surprisingly, they re-entrain to an advance of the LD cycle by daily phase advances of ~ 1 h magnitude (Fig 3d), and to a delay of LD cycle by a series of ~ 1 h daily delays. By contrast, Norway rats typically express a $\tau > 24$ h, and re-entrain to an 8 h LD advance by phase delaying 16 h (Mistlberger, unpublished data). Endogenous τ in humans is slightly longer than 24 h, which likely contributes to more rapid re-entrainment after flying west (requiring a delay) than east (requiring an advance), and explains why humans may phase delay for many cycles to achieve a large phase advance (18).

The experimental analysis of entrainment mechanisms in mammals has been based primarily on a few nocturnal rodent species that express very precise circadian rest-activity rhythms, enabling accurate measurement of even small phase shifts. Relatively little work has been done on diurnal mammals. Unlike their nocturnal cousins, diurnal animals are typically exposed to light for long durations each day. However, light pulse PRCs in the few diurnal mammals studied so far appear to be qualitatively similar to those for nocturnal rodents (e.g., 19). Thus the mechanism of 'discrete' entrainment by daily phase adjustments induced by light exposure at dawn and dusk appears to be available to diurnal as well as nocturnal species.

Evidence from studies of the European ground squirrel, however, suggest that in the natural environment this 'discrete' entrainment may be only part of the entrainment mechanism in diurnal animals (20). In its natural environment, this semi-fossorial diurnal rodent completely avoids dawn and dusk; it spends the night in a dark, enclosed burrow, emerges to forage ~ 4 h after sunrise, returns to its burrow ~ 3 before sunset, and blocks the burrow entrance with soil before retiring to sleep. Light-exposure is therefore self-selected and falls during the predicted dead-zone of the photic PRC. Under these circumstances the circadian clock should free-run, yet these animals exhibit a stable diurnal phase of entrainment without systematic drifts toward dawn or dusk. It is not yet clear how this is accomplished, but it suggests that light exposure during the day is modulating τ .

Recent studies of the human circadian pacemaker also indicate that it is sensitive to light throughout the subjective day (21), apparently exerting a continuous action on the pacemaker. Entrainment might be accomplished without discrete phase shifts during lighting transitions by accelerating and/or slowing the angular motion of the pacemaker throughout the day, with the net result of a 24 h τ and stable Ψ_{LD} . This model of a continuous action of light on pacemaker τ forms the basis of a 'continuous' or 'parametric' model of entrainment.

Support for this model derives from the observation that τ varies with the intensity of constant light (LL) exposure in most species (22). In nocturnal rodents, LL slows the circadian clock (i.e., τ is lengthened), shortens the daily active phase and reduces total daily activity (e.g., Fig. 5d). In diurnal animals, LL generally has the opposite set of effects, although there are more exceptions across species (and diurnal primates, in particular, tend not to conform). These empirical generalizations are collectively known as the 'Circadian Rule' (22) and suggest that light could facilitate entrainment by continuous modulation of τ . While this mechanism is not applicable to semi-fossorial nocturnal species that see little daylight, it may contribute to entrainment of diurnal species. It may also account for entrainment in animals at high latitudes that are exposed during mid-summer to continuous illumination with a sinusoidal variation in intensity. Modulation of τ in diurnal animals by total daily light may also have consequences for Ψ_{LD} , which would be particularly apparent at the extremes of winter and summer photoperiods.

It is also possible that extended daily light exposure in diurnal animals affects the amplitude of the circadian clock. A widely used model of the circadian clock treats it as a limit-cycle oscillator that has both phase and amplitude dimensions, and a preferred trajectory of oscillation between two or more state variables. When the phase of the oscillation is acutely shifted, the amplitude of oscillation may also transiently change, before the preferred trajectory (the limit cycle) is re-established. A continuous or very strong zeitgeber (e.g., critically timed bright light) may drive the state parameters to a low amplitude trajectory, or even a point of singularity characterized by zero amplitude. When the amplitude of oscillation is small, a resetting stimulus of given intensity will elicit larger phase shifts (23,24). Such effects may underlie potentiated responses to phase-shifting stimuli in Syrian hamsters after exposure to 2 days of LL (25,26). If the long photoperiod of summer at high latitudes does affect the amplitude of the circadian clock, then this may also affect the Ψ_{LD} at which entrainment stabilizes. Such effects remain to be empirically substantiated.

Another influential heuristic model of the circadian clock postulates that the master, light-entrainable circadian pacemaker consists of two coupled oscillators, each with a full or partial PRC to light (13; in mammals, this clock is located in the hypothalamic suprachiasmatic nucleus; SCN, see chapter xx). When entrained to a natural photoperiod, the two oscillators are assumed to be coupled with a phase relation such that one is affected primarily by morning

light, and the other primarily by evening light. This dual-oscillator model accounts successfully for many features of circadian rhythms observed under skeleton and non-24 h LD cycles, and in response to LD shifts and light pulses.

Although the model was developed to explain photic entrainment and photoperiodic timing of annual rhythms, it was inspired by the observation that animals exposed to constant lighting conditions for several weeks often exhibit splitting of their circadian rhythms (including those of activity, temperature and hormones) into two components. In nocturnal hamsters, these features emerge after exposure to bright LL for many days. The two split components often free-run with different τ 's for at least a few cycles before coupling stably in an antiphase relation (e.g., Fig. 4a). The components rapidly revert to normal coupling under standard photoperiods, but can be maintained in the split state by certain exotic photoperiods (27). A two-oscillator organization appears to generalize to at least some diurnal organisms, as perhaps the best examples of prolonged splitting are seen in diurnal tree shrews maintained in DD (28). The two-oscillator formalism continues to stimulate debate and experiment in search of its cellular and molecular basis (29,30; see also chapter xx).

Although early evidence suggested that the circadian clock in humans was especially sensitive to social cues and less so to photic cues, it is now clear that humans can be entrained by LD cycles and exhibit phase shifts to light pulses with a PRC qualitatively similar to that for other organisms (31). These and other properties of circadian regulation in humans are described in greater detail in Chapters x and x).

B. Nonphotic Entrainment

1. Masking and Entrainment

An important distinction in chronobiology is that between 'masking' and 'entraining' effects of environmental stimuli. Light can entrain circadian rhythms in virtually every species that has been studied carefully. In many species, light also has direct effects on the expression of behavior and physiology. In nocturnal rodents, light at night inhibits locomotor activity and promotes sleep. Light in this case constitutes a 'negative masking' stimulus, because it suppresses a behavior at a circadian phase when that behavior is usually present, thereby 'masking' the true phase of the circadian clock. An example of partial negative masking is illustrated in Fig. 3 (panels d and e); activity early or late in the hamster's subjective night is strongly suppressed by light exposure following a delay or advance, respectively, of the LD cycle. In diurnal animals, light usually stimulates activity; at night this would constitute a 'positive' masking effect. Another well-known masking effect of light is acute suppression of nocturnal pineal melatonin secretion (32), a rapid response that is evident in nocturnal and diurnal animals.

These direct effects of environmental stimuli on physiology and behavior contribute to the waveform of daily rhythms in natural environments. They can also present interpretive difficulties in evaluating whether a periodic environmental stimulus functions as a true zeitgeber (i.e., affects the

pacemaker rather than only overt behavior). A periodic signal may entrain a behavioral or physiological rhythm, in which case it is functioning as a zeitgeber, or it may directly impose a rhythm without affecting an underlying oscillator, in which case it is functioning as a masking stimulus. The criteria for distinguishing masking from entrainment are based on the properties of oscillator entrainment discussed in relation to photic cycles.

- An entrained rhythm should persist (free-run) in the absence of the periodic stimulus, just as photically entrained rhythms persist in constant lighting conditions, and the initial phase of the free-run should reflect the apparent phase of the rhythm during entrainment.
- An entrained rhythm should follow an environmental cycle over only a limited range of environmental periods.
- The phase relation between an entrained rhythm and an environmental cycle should vary depending on the values of τ and T .
- This phase relation should be re-established if the environmental cycle is abruptly shifted. If the rhythm is a direct response to the environmental cycle, shifting will be immediate. If, instead, it reflects entrainment of an underlying oscillator, several transient cycles may be evident before the prior phase relation is re-established.

As reviewed in the following sections, several nonphotic stimuli have been identified that meet these criteria and can therefore be said to entrain circadian rhythms.

2. Scheduled Feeding

Rats provided with food for only a few hours each day at a fixed time show changes in locomotor activity, body temperature and other physiological variables that anticipate the daily mealtime (Mistlberger, 1994). These anticipatory responses begin to emerge within several days of scheduled feeding, and appear to be generated independently of photically entrained rhythms. If the meal is provided in the middle of the light period, rats exhibit two main bouts of activity each day, an intense bout prior to mealtime, and at least some activity persisting during the usual nocturnal active period (Fig. 4b). If the photic cycle is replaced by LL or DD, photically entrained rest-activity rhythms free-run while pre-meal activity remains coupled to mealtime (Fig. 4c). It is common, then, to observe two circadian activity components in food-restricted rats in constant lighting, one free-running with a period slightly different from 24 h, and a second, food-anticipatory component with a period of exactly 24 h.

Several models have been proposed to account for the emergence of food-anticipatory activity rhythms (33). Most findings, however, are explained parsimoniously by the hypothesis that anticipatory activity is generated by circadian oscillators entrained by food availability:

- Anticipatory responses do not emerge when the period of the food cycle is outside a broad circadian range of about 22 to 33 h (34).

- The phase relation between anticipatory activity onset and mealtime becomes increasingly positive as the period of the feeding cycle is lengthened and less positive as it is shortened (35).
- Phase shifting the feeding time is usually followed by gradual rather than immediate shifts of the activity peak to resynchronize to the mealtime (36).
- When the period of the food availability cycle is abruptly shortened or lengthened from 24 h to 22 h or 30 h, the meal-synchronized rhythm may dissociate from feeding time and free-run for many cycles (34).
- When food is made freely available, the control exerted by these oscillators over behavior diminishes, resulting in a loss of overt activity related to the former mealtime. However, when the rat is subsequently food deprived for several days, activity reappears at the circadian phase previously associated with feeding time (37).

Taken together, these observations provide strong evidence that a self-sustained oscillator is involved in generating meal-anticipatory activity, and indicate that its influence on behavior is gated by the animal's motivational state. Other studies show that rats can utilize food-entrainable oscillators to discriminate time of day, which may enable them to anticipate multiple feeding times and link these with multiple feeding places (9). Dissociation of food-anticipatory rhythms from LD-entrained rhythms suggests separate food- and light-entrainable circadian clocks, and lesion studies have confirmed that the master light-entrainable circadian pacemaker (the suprachiasmatic nucleus) is not necessary for food-entrained rhythms. Although food-anticipatory behavioral rhythms have been described in numerous species, (reviewed in 33) only in rats, hamsters and mice have the appropriate studies been done to confirm mediation by a separate food-entrained clock (38–40). The broad physiological significance of such food-entrained rhythms may be related to the discovery that some peripheral organs and tissues express genes with a circadian rhythm that is entrained preferentially by feeding cycles rather than LD cycles (41,42) (see chapter xx). However, the site of the circadian mechanisms that drive food anticipatory behavioral rhythms has not yet been identified.

The stimuli associated with food acquisition and ingestion that provide the timing signals necessary to entrain circadian oscillators are also not well characterized. Rats can anticipate meals of any nutrient composition, provided that these meals represent a significant contribution to daily caloric intake (43,44). Similar results have been obtained in studies examining the ability of specific nutrients to shift food anticipatory rhythms (45). Observations of anticipatory behaviors to restricted schedules of water (46) or salt (48) access suggest that the range of effective stimuli may extend to any motivationally significant resource for which a rat must forage.

The use of separate pacemakers to mediate food and light entrainment in rats confers flexibility on the circadian organization of their behavior, and permits anticipation of a stable window of food availability at any time of day, without necessarily altering the phase of photically entrained activity rhythms.

However, occasionally feeding schedules do entrain or markedly shift the entire circadian system in rats (49) and in some species this may be the rule rather than the exception (e.g., Fig 4e) (40, 50, 51, 52). In these cases, it is not clear which stimuli associated with scheduled feeding serve as the zeitgeber for the light-entrainable circadian clock in the SCN. The SCN clock may, for example, be entrained indirectly as a result of its coupling relations with food-entrained oscillators located elsewhere. Alternatively, the SCN may be entrained by stimuli from specific ingested nutrients, metabolic hormones associated with their ingestion, or non-specific stimuli, such as the arousal associated with anticipatory activity or the thermogenic consequences of digestion. The following sections consider the evidence that non-specific, non-photic stimuli can entrain circadian rhythms in mammals.

3. Temperature

Free-running rhythms in a variety of heterothermic species can be entrained by 24 h cycles of ambient temperature (53). However, responses of homeothermic animals to temperature cycles are more variable. In some species, temperature cycles failed to entrain various circadian rhythms, (54,55) whereas in other species, including squirrel monkeys (56), pig-tailed macaques (57), rats and a marsupial mouse (58), temperature cycles have clear entrainment effects in at least some individuals.

The mechanism by which temperature entrains circadian rhythms in mammals is unclear. The SCN circadian pacemaker, studied in vitro, can be phase shifted by temperature pulses (step changes from 34–37°), possibly by a direct effect on clock gene transcription or translation (59). In homeothermic species in vivo, however, entrainment to temperature pulses would seem unlikely to involve a direct effect on circadian oscillators in the brain, assuming that brain temperature is defended homeostatically. However, some mammals can regularly sustain very low body temperatures, and in these cases significant changes in pacemaker temperature may have τ modulating and phase resetting effects (60). It is also possible that temperature cycles affect the circadian clock indirectly, by inhibiting or facilitating sleep and wake. The neural and endocrine correlates of changes in sleep-wake states, and the obvious changes in food and water intake, activity, and light exposure they entail, may in turn have phase-modulating feedback effects on circadian oscillators. Conceivably, any stimulus that has potent effects on behavioral state may have significance as a circadian zeitgeber.

4. Activity and Arousal States

In an early description of the Circadian Rule, Aschoff noted that the relation between light intensity and τ might be mediated by changes in arousal states, since bright LL both lengthens τ and suppresses activity in nocturnal rodents (22). A later study provided some support for the idea that metabolism, arousal or some correlate affected the circadian pacemaker, in that the free-running τ of activity rhythms (measured as general locomotor activity

using photocell interruptions) was longer in hamsters with access to running wheels (61). More recent studies indicate that this effect is weak in hamsters (62), but is robust in rats (63) and mice (64): τ shortens when wheels are available, and lengthens when wheels are locked. Evidently, some correlate of spontaneous, clock-regulated wheel-running activity provides a feedback signal that alters the rate at which the circadian pacemaker cycles.

The effects of behavior on functional properties of the circadian pacemaker were not widely recognized until the late 1980's, when a series of studies demonstrated unequivocally that acutely stimulated locomotor activity and arousal can reliably induce large phase shifts in hamsters (Fig. 5a,b), and, if repeated on a daily basis, can entrain free-running rhythms in hamsters, rats and mice (65–69). The earliest of these studies showed that hamsters could be phase-shifted or entrained simply by changing their litter or cages (65) or by the opportunity for a 30 min daily bout of foraging activity in an open field, despite little or no eating at that time (66). More robust phase shifts were reliably obtained by confining hamsters in DD to novel running wheels for 2 to 3 h. Hamsters that ran continuously while in the wheel exhibited phase shifts of 2–3h or more (67).

As with phase-shifting light stimuli, the magnitude and direction of the phase shifts evoked by activity depend on when the stimulus is scheduled. The shape of the associated PRC, however, is markedly different; running stimulated during the mid-subjective day (the 'dead-zone' for photic stimuli) induces large phase advance shifts, whereas running during the mid-to-late subjective night (the advance zone for photic stimuli) induces small phase delays (Fig. 5c). These findings indicate that the circadian clock in mammals has distinct circadian rhythms of sensitivity to photic and nonphotic stimuli.

A variety of stimuli have been shown to induce phase shifts by stimulating activity; these include 3–6 h 'pulses' of darkness interrupting LL, injections of triazolam or morphine, cold exposure, and activity stimulated by an acute bout of food deprivation (68–71). All of these stimuli also necessarily arouse animals, raising the possibility that phase shifts are due to being awake, rather than to intense or continuous running. Indeed, in Syrian hamsters, the phase-shifting effects of stimulated running can be fully mimicked by the sleep deprivation procedure of gentle handling, which is associated with only low levels of occasional activity in the home cage (72). However, if locomotor activity is entirely prevented, by confining hamsters to a restraint tube, or to a small platform over water, no phase shifts occur, even if arousal is confirmed by concurrent EEG or plasma cortisol measures (71,73). Thus, locomotion does appear to be necessary for the induction of phase shifts in response to arousal procedures. It has not yet been definitively ruled out that neuroendocrine correlates of the stress of these procedures actually inhibit the phase-shifting effect of being awake in the usual rest period.

The conclusion that "non-photic" stimuli phase shift because they induce activity is clearly not universally applicable to all stimuli, nor to all species. Dark pulses, for example, phase shift sparrows even though darkness inhibits their

activity (74) and shift rhythms of pineal cells cultured in vitro, (75) where the issue of mediation by activity is not relevant. Dark pulses in the 'subjective night' in hamsters can also induce shifts by an apparent 'anti-photic' mechanism independent of locomotor activity (76). A variety of drugs have also been shown to induce phase advances during the subjective day that are either not associated with increased activity or are not blocked by procedures for preventing activity. These pharmacological manipulations may directly activate pathways (e.g., serotonin, neuropeptide Y) that mediate the effects of activity and arousal on the circadian clock (76, 77).

The adaptive significance of the phase-shifting effects of activity in hamsters is unclear, but it may represent a mechanism for modulating circadian activity patterns in response to biologically important events related, for example, to social or sexual interactions (for review, see 78). The potential practical applications of this phenomenon are, however, notable. For example, a single 3 h bout of appropriately scheduled wheel activity in hamsters can greatly accelerate re-entrainment to a shifted LD cycle (79) (compare Fig. 3d with Fig. 5d), and repeated daily bouts can alter the phase of entrainment to an LD cycle (80,81). Scheduled activity can shift circadian rhythms in humans, and may thus represent a useful tool for manipulating the circadian system to remedy acute disorders associated with travel across time zones, shift work (82) or disorders of entrainment, such as are commonly experienced by the blind (83).

5. Social Cues

Interactions between members of the same species are known to phase shift, entrain or modify the period of free-running rhythms in several species (78). However, interpreting these effects can be problematic, as social interactions can have strong masking effects and may also determine the pattern of light exposure, thereby complicating attributions of causality. In most cases, social effects on circadian timing appear to be weak, and in many experiments, no effects were observed. For example, under standard laboratory housing, the circadian rhythms of rats, mice and hamsters housed individually in adjacent cages typically do not become mutually synchronized despite auditory, olfactory, and visual contact. Hamsters housed in the same cage may fail to become mutually entrained (84), although co-housing a resident dominant hamster with a subordinate for 3 h in the middle of the usual rest period can result in very large phase advance shifts in one or both of the pair (73). Blind humans may also display free-running sleep-wake and other rhythms despite exposure to the social cues of a 24 h society (83). The mechanisms by which social cues influence circadian timing are not known, but effects on arousal states are probably a necessary, if not sufficient, component of this mechanism (78).

C. Interactions between photic and nonphotic zeitgebers

The striking effects of spontaneous activity on free-running τ in rats and mice, and of stimulated activity on phase in free-running hamsters, suggest that the steady-state, entrained phase of circadian rhythms may reflect integration of photic and nonphotic stimuli. A number of studies, most using hamsters as subjects, have examined whether activity stimulated each day at a particular phase of the LD cycle can alter entrainment to LD cycles. The results of some of these studies suggest that photic and nonphotic inputs combine linearly, in accordance with their respective PRCs. For example, activity or arousal induced in the middle of the light period by triazolam injections, wheel confinement, social/sexual stimuli, or restricted feeding can advance the phase of nocturnal activity onset (50, 77), whereas activity induced late in the subjective night can delay the phase of activity onset (73,80).

Other results indicate that under some circumstances photic and nonphotic stimuli may combine non-linearly, and produce phase changes that are not predictable from separate analyses of the photic and nonphotic PRCs. Two studies have shown that activity induced in the middle of the light period can in some cases significantly delay, rather than advance, nocturnal activity onset (81,83). This apparent delay may reflect splitting of the activity rhythm into two components, one that delays with respect to dark onset, and another that advances and aligns with the mid-day wheel-running session (27,81,85).

Other studies have examined the interactive effects of single pulses of photic and nonphotic stimuli in combination (for detailed review and references, see 77). The results again suggest complex, non-linear interactions. Phase advances induced by an activity bout in the mid-subjective day can be blocked by a subsequent light pulse, although the light pulse alone at that time produces no phase shift. Conversely, phase advances induced by a light pulse in the late subjective night can be significantly attenuated by a 45 min bout of stimulated running, which alone does not induce phase shifts at this phase. Phase delay shifts to light early in the subjective night were not inhibited by concurrent running, but were attenuated by a 6 h sleep deprivation procedure, in both hamsters and mice. Thus, all portions of the photic PRC are amenable to inhibition by manipulations of behavior, although the delay and advance zones may be differentially sensitive.

In other combinations, photic and nonphotic stimuli can show non-linear synergy rather than mutual inhibition. Hamsters subjected to a 7 h advance of the LD cycle re-entrained within 1 or 2 cycles if they were induced to run for 3 h at the beginning of the new dark period, as compared to 6–10 days if they were left undisturbed (Fig 5d) (79). The rapidity of phase shifting in this paradigm is clearly greater than that predicted by simple addition of the photic and nonphotic PRCs. The analysis of the rules of interaction between photic and nonphotic zeitgebers is still at an early stage but should benefit from progress made on the molecular basis of circadian clock resetting.

The study of nonphotic effects on circadian rhythms in the past decade has produced a number of surprising discoveries, one of which is the report that

a neutral, nonphotic stimulus can acquire the phase-shifting properties of light by repeated pairing with light pulses. An air disturbance created by a fan that reliably preceded a daily, phase-shifting light pulse, was reported to acquire the capacity to induce a phase shift similar to that induced by light in rats (86). While attempts to replicate this finding using modified conditions have not yet been successful (87), it remains possible that both current and previous exposure to nonphotic stimuli can modify photic entrainment processes. Other studies have, for example, demonstrated that photic phase shifts can be inhibited in an environment previously conditioned to evoke fear (88). The neurochemical interactions that underlie such effects may be related to the mechanisms by which non-photic and photic stimuli interact in the absence of conditioning.

IV. Circadian Rhythms across the lifespan

Assessment of biological rhythmicity across the mammalian lifespan presents many methodological and interpretive challenges. Nevertheless, a valid cross-species generalization is that circadian rhythms in behavioral, physiological or neural functions are first evident prenatally, stabilize and entrain to LD cycles within the first few weeks (e.g., in rodents) or months (e.g., in humans) postnatally, and may exhibit changes in phase, period, amplitude and response to zeitgebers in old age, with considerable variability within and among species.

In rodents, behavioral rhythmicity is not apparent before the second week postnatally (89, 90). Extrapolation of the phase at which behavioral and physiological rhythms emerge back to prenatal life indicates, however, that the circadian pacemaker is functioning at or before birth in rats, hamsters and mice. 2-Deoxyglucose autoradiography has been used to confirm that the circadian pacemaker located in the SCN expresses a daily rhythm of glucose metabolism as early as day 19 of gestation, which is 2–3 days before birth and prior to significant synaptogenesis in the SCN (91). Although the fetal pacemaker lacks most input and output pathways, its metabolic rhythm is synchronized to the dam by a mechanism that involves pacemaker sensitivity to endogenous dopamine and maternal melatonin. Postnatally, the pacemaker becomes sensitive to photic stimuli during days 2–6, at which time it loses sensitivity to dopamine (92). Maternal coordination of the fetus in utero ensures that when the pacemaker becomes coupled to effector systems for behavior and physiology postnatally, the emerging rhythms in these functions are appropriately phased with respect to the day–night cycle, and to rhythms of the dam.

Studies of the human fetus in utero have revealed daily variations in fetal motility, heart rate and other variables by 20–22 weeks gestation (93). Although these rhythms may be driven directly by maternal factors, evidence for circadian periodicities of various functions in premature infants suggests that humans can generate rhythms endogenously as early as 30 weeks of gestation (94). Neonates tracked over the first year of life exhibit a gradual emergence of circadian rhythmicity which initially may appear to free-run, but

which generally becomes synchronized to the day-night cycle within 2–3 months. The conditions under which infants are maintained (e.g., on demand vs. scheduled feeding) can greatly influence the temporal organization of sleep-wake states (reviewed in 93). The extent to which maturation of masking and entrainment processes contribute to the appearance of synchronized rhythms early in life is unknown.

Poor sleep is a primary concern of the aged, and deterioration of circadian organization may be a significant contributing factor. The most widely observed change in circadian rhythmicity with age is a decrease in the amplitude of a variety of rhythms, including activity, temperature, and sleep-wake, under entrained and free-running conditions (95). However, a reduced rhythm amplitude is difficult to interpret, and could reflect changes in intrinsic properties of the pacemaker (e.g., coupling among a population of cellular oscillators), or changes upstream or downstream from the molecular machinery of the circadian clock. Changes upstream from the pacemaker may include reductions in sensitivity to photic or nonphotic stimuli. There is evidence that phase shifts to light at low intensities are reduced in aged rodents, but that responses to saturating intensities are greatly enhanced, at least at the transition point between delays and advances on the photic PRC (96). These effects are consistent with a reduced sensitivity to light at the receptor level, and reduced amplitude of oscillation at the pacemaker level. Phase-shift responses to nonphotic stimuli (e.g., triazolam injections, dark pulses) have also been reported to be reduced in aged rodents in some studies (96), but not others (97), and may to some extent be secondary to a reduced running response to the stimulus in old animals.

Daily rhythms of sleep in aged humans are commonly viewed as ‘fragmented’, with less sleep at night and more napping during the day (98). A reduced amplitude of circadian rhythms has been reported in entrained and free-running humans in many but not all studies (95). Failure to observe differences between young and old subjects in some samples may reflect individual differences and illustrate limitations of cross-sectional studies, because these of necessity include only survivors in the advanced age groups, whose circadian mechanisms may differ from those of others in the cohort. In addition, the unknown histories of light exposure and entrainment of subjects in these studies may contribute significantly to observed differences.

Another commonly observed effect of aging in rodents and humans is an advanced Ψ_{LD} (95). This is consistent with reports that free-running τ shortens with age. This effect is consistently evident in studies employing extended longitudinal, rather than cross-sectional, designs (e.g., 99). In other species τ may lengthen with age (e.g., 100). Detection of τ changes may depend on the use of sufficiently aged subjects (many studies have used middle-aged subjects), on the duration of recording (after-effects of previous photoperiods may obscure age-related changes) and on the recording method. In rats and mice, for example, wheel-running activity shortens τ and its intensity is significantly attenuated with age. Any possible changes in τ intrinsic to an aging

pacemaker might thus be obscured by changes secondary to a reduction of feedback signals from behavior, arousal, hormones or other factors.

It has been suggested that loss of temporal order may be a contributing cause, rather than merely a consequence, of aging (101). In aged rodents, loss of circadian rhythmicity is predictive of impending death (102) and extended exposure to lighting schedules at the limits to entrainment can reduce lifespan in some invertebrate species (103). Although loss of rhythmicity due to experimental ablation of the SCN circadian pacemaker is clearly not fatal to laboratory rodents, effects on mean and maximal lifespan have not been studied carefully. The empirical database remains insufficient to draw strong conclusions about causal relations between altered circadian organization and the normal aging process.

V. Conclusion

This overview of the properties of circadian regulation of behavior and physiology highlights the complexity of the underlying circadian system. The circadian system in mammals is sensitive to light and nonphotic cues, and manipulations of these have revealed a multiplicity of circadian clocks and complex interactions among inputs to these clocks. Black-box models of the circadian system based on a vast descriptive literature have provided a framework for analyses at the neurobiological and molecular level, and progress at these levels has been remarkable over the past decade. This work is reviewed in detail in chapters xx and xx.

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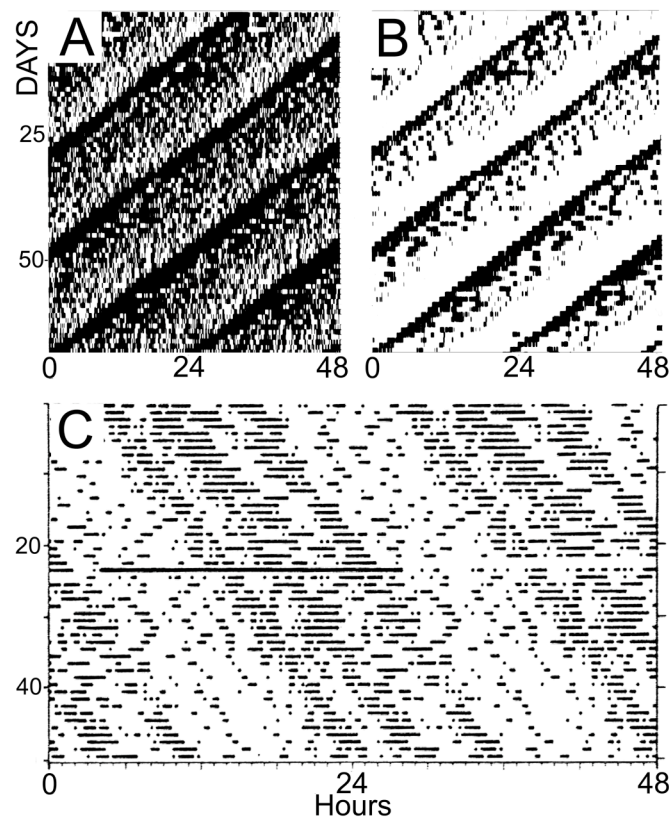
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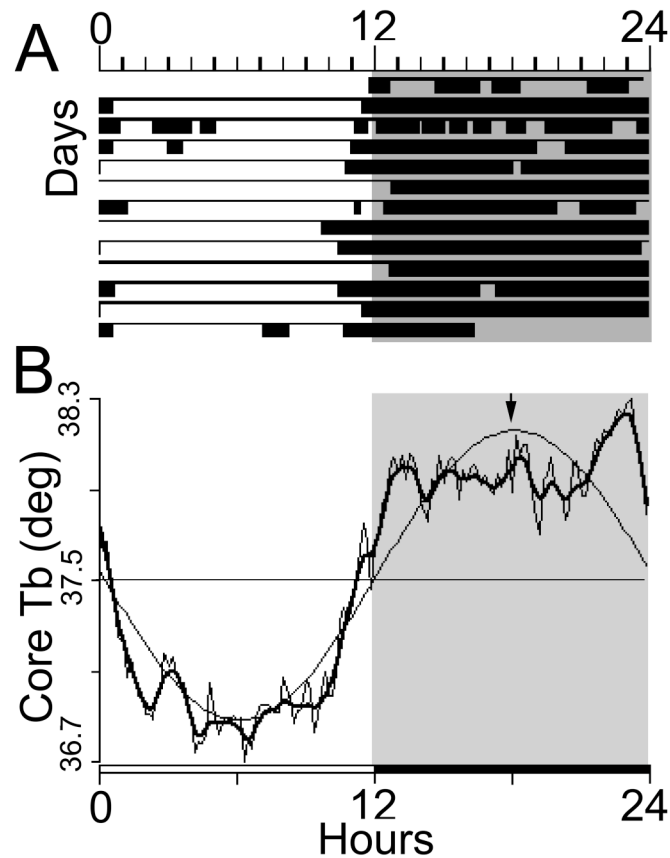
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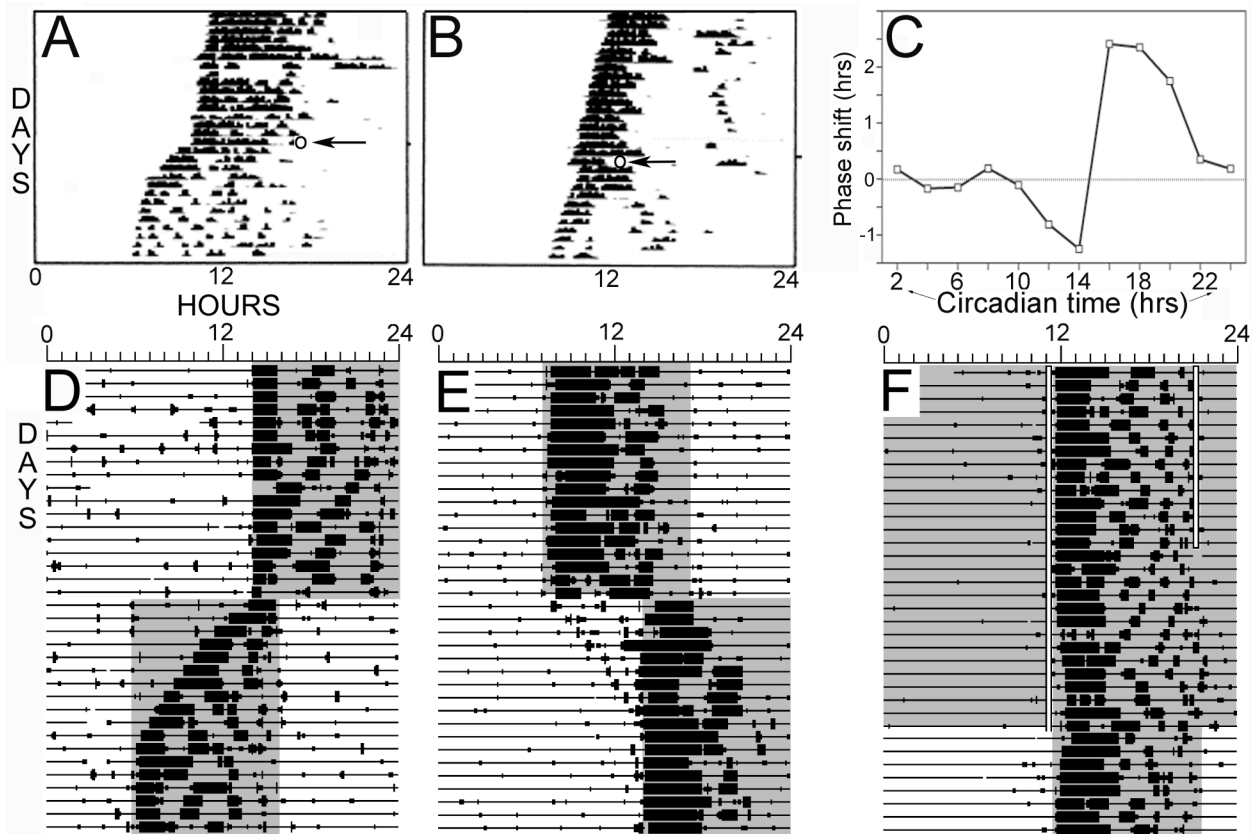
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Figure 1.

Circadian rhythms ‘free-running’ in constant dark, plotted in conventional ‘actogram’ format. A. The circadian rhythm of polygraphically recorded wake time in a C57 mouse. Each line represents 48 consecutive hours, and consecutive days are aligned vertically (thus the data are double-plotted, i.e., the right half of the chart is a duplicate of the left half, but is moved up one line). Data were collected in 6 min time bins, and a black bar was assigned to those bins during which wake time accounted for more than 50% of the 6 min. This plotting format reveals at a glance that there is a bout of continuous waking lasting 2–3 h each circadian cycle, and that this bout occurs a bit earlier each 24 h day, i.e., the circadian cycle is <24 h. B. The circadian rhythm of wheel-running activity from the same mouse. Note the precise onsets of the daily active period. In rodents, all circadian rhythms free-run in phase. C. The circadian rhythm of nonspecific locomotor activity in a rat, measured by photobeams. Note that activity onsets occur a little later each day; i.e., the circadian cycle is >24 h. The long black line on day 23 denotes a 24 period of forced activity in a rotating drum; this procedure did not perturb the free-running rhythm. (A,B. Modified from Welsh DK, Engel EM, Richardson GS, et al: Precision of circadian wake and activity onset timing in the mouse, *J Comp Physiol [A]*. 1986;158:827–34. C. Modified from Borbely AA: Sleep regulation: circadian rhythm and homeostasis, *Curr Topics Neuroendocrin*. 1982;1:83–103.)

Figure 2.

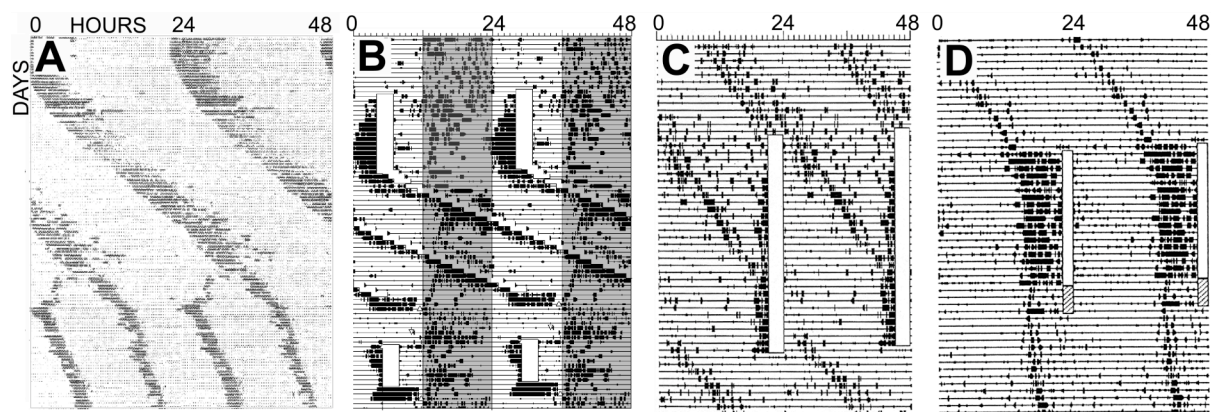
A. Circadian rhythm of core body temperature recorded from a rat by telemetry. The data are single-plotted (each line is 24 h, with 5 min bins plotted left to right, and consecutive days aligned vertically). The interval with lights-off (12 h) is denoted by the shading. B. Average waveform constructed from the data in panel A. The thin line represents the raw data and the heavy line a running average. A cosine function was fit to the smoothed data, and the arrow denotes the peak (acrophase) of the function. Note that the waveform of the temperature rhythm in LD has a prominent square wave component, evident particularly during the nocturnal segment; thus, a cosine function is not an optimal fit, as the acrophase occurs ~6 h before the true peak in the raw data. (Mistlberger, unpublished data)

Figure 3.

Phase shifting and entrainment by light. A. Circadian rhythm of wheel running recorded from a hamster in constant dark. On the day indicated by the arrow, the hamster was exposed to light for 30 min (open circle). The pulse was timed to occur at circadian time 20, which is 8 circadian hours (where 1 circadian hour = $\tau/24$) after the daily onset of wheel running (denoted circadian time 12, by convention, and estimated by fitting a regression line to the activity onsets for 7–10 days prior to the day of light exposure). On the day after the light pulse, activity occurs earlier than predicted by extrapolation from the preceding free run. After several days of ‘transient’ advancing cycles, a stable phase advance shift of several hours is revealed. Transients are by definition those cycles prior to completion of the full shift. B. In this experiment, the light pulse was presented at circadian time 14. This induced a small phase delay shift of ~1 h, which was complete within one circadian cycle. Phase resetting without transients is typical of light-induced delay shifts. C. A full phase response curve depicting the relation between the circadian time of light exposure, and the magnitude and direction of the resulting phase shift. Delay shifts occur to light early in the subjective night (~CT10–15) and advance shifts to light late in the subjective night (CT15–24). D. 8 h phase advance of the LD cycle, illustrating gradual re-entrainment by a series of ~1 h phase advances each day (due to differential exposure of the delay and advance portions of the circadian

pacemaker's PRC to light). Note inhibition of activity by light during the later portion of the hamster's active period, during the first few days after the LD shift. E. 6 h phase delay of the LD cycle, illustrating gradual re-entrainment by delay shifts. Note inhibition of running early in the hamster's active period. F. Stable entrainment to a skeleton photoperiod in a hamster exposed to two daily 30 min pulses of light (denoted by the vertical bars), simulating dawn and dusk and defining a 14 h 'day' (diurnal rest period) and 10 h 'night' (nocturnal active period). The hamster's activity rhythm began to phase delay when the 'morning' light pulse was omitted, and a stable phase was restored when a full 14:10 photoperiod was implemented. (C. modified from Takahashi JS, DeCoursey PJ, Bauman L, et al: Spectral sensitivity of a novel photoreceptive system mediating entrainment of mammalian circadian rhythms, *Nature* 1984;308:186-188. D. modified from Mistlberger RE, Nadeau J. Ethanol and circadian rhythms in the Syrian hamster: Effects on entrained phase, reentrainment rate and period. *Pharm Biochem Beh.* 1992;43:159-165. E and F. Mistlberger, unpublished).

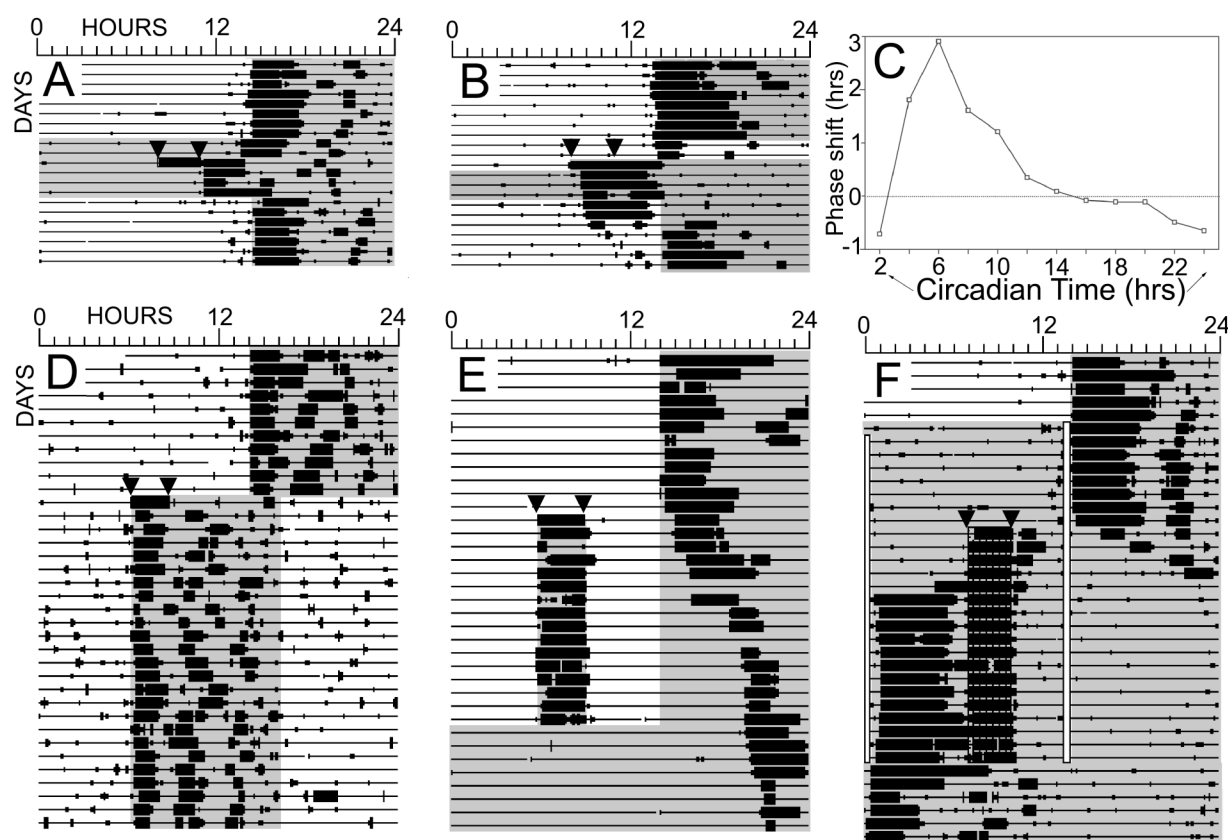
Figure 4.



A. Wheel running activity of a Syrian hamster in constant light. The rhythm 'splits' into two components that free-run with different periods for about 10 days, then couple in 'antiphase'. Note that the period of the free-running rhythm shortens after the split, suggesting that τ in the coupled state depends on the phase relation between two underlying oscillators. B. Wheel-running rhythm of a rat entrained to a 12:12 LD cycle during ad lib feeding, restricted feeding (open vertical bars) and food deprivation. Running is primarily nocturnal during ad lib feeding. A prominent bout of running anticipates a 3 h daily mealtime, and remains coupled to mealtime when feeding intervals are extended from 24 to 26 h. Activity persists at the predicted mealtime when food is withheld for 3 days (note heavy bouts of daytime running, and rapid reversion to nocturnal activity when food is restored). C. Free-running rhythm of a rat in constant light does not entrain to a restricted 3 h daily mealtime, despite anticipatory activity. These data suggest that there are separate pacemakers driving free-running (light-entrainable) circadian rhythms and food-anticipatory

rhythms. D. Free-running activity rhythm of a hamster in constant light entrains to a restricted daily feeding and drinking opportunity. (A. Pickard GE, Turek FW: The suprachiasmatic nuclei: two circadian clocks? *Brain Res.* 1983;268:201–10. B. Mistlberger RE, Marchant EG: Computational and entrainment models of circadian food-anticipatory activity: Evidence from non-24 h feeding schedules, *Behav Neurosci.* 1995;109:790–798. C. Mistlberger, unpublished. D. Mistlberger RE: Effects of scheduled food and water access on circadian rhythms of hamsters in constant light, dark, and light:dark, *Physiol Behav.* 1993;53:509–516.)

Figure 5.



Phase shifting effects of 3 h bouts of arousal stimulated by confinement to a novel running wheel during the middle of the subjective day (rest period) in Syrian hamsters. A. 150 min phase advance induced by running on the third day of constant dark. B. 350 min phase advance induced by running on the third day of constant light. Lights were turned off during running and left off for the next 3 days. C. Phase response curve to 3-h bouts of wheel running. D. 8-h phase shift of LD, combined with 3 h of stimulated running at the onset of darkness on the first night of the advanced LD cycle. The hamster re-entrains to LD within 1 cycle (compare this with Figure 3D, same hamster without

stimulated running). E. Phase delay of nocturnal activity in a hamster entrained to LD and subjected to novelty-induced running each day. F. Inversion of the circadian activity rhythm in a hamster entrained to a skeleton photoperiod (two 30 min light pulses) and subjected to novelty induced running. Such inversions are prevented by full photoperiods. (A, B: Mistlberger RE, Antle MC, Webb IC, et al: Circadian clock resetting by arousal: The role of stress and activity, *Am J Physiol.* 2003;285:R917–25. C. modified from Mrosovsky N, Salmon PA, Menaker M, et al: Nonphotic phase-shifting in hamster clock mutants, *J Biol Rhythms* 1992;7:41–49. D. modified from Mistlberger RE, Nadeau J: Ethanol and circadian rhythms in the Syrian hamster: Effects on entrained phase, reentrainment rate and period. *Pharm Biochem Beh.* 1992;43:159–165. E. Sinclair SV, Mistlberger RE: Activity reorganizes circadian phase of Syrian hamsters under full and skeleton photoperiods, *Behav Brain Res.* 1997;87:127–137.