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Physiology and regulation of biological rhythms in laboratory animals: an overview

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Summary

Biological rhythms have been observed in practically all groups of laboratory mammals and at every level of physiological and behavioural organization. Biological rhythms are classified according to their period as ultradian (<24 h), circadian (~24 h), infradian (>24 h), and seasonal or circannual rhythms (~1 year). This review outlines what is known about the neurobiology of biological rhythms in mammals and describes the hierarchical order in which ultradian, circadian and infradian rhythms are related to each other. The article does not attempt to catalogue every physiological variable showing rhythmical fluctuations in laboratory mammals. Rather, it focuses on the basic concepts of circadian rhythms and recent advances made in our understanding of the physiology of the internal clock controlling circadian and other biological rhythms.

Keywords: Ultradian; Circadian; Infradian; Seasonal, and Circannual Rhythms; Photoperiodism

Many characteristics of laboratory animals vary more or less regularly with specific periods. These temporal variations are called biological rhythms and can be observed not only in the whole organism but also in single organs, tissues or even isolated cells. Biological rhythms cover a wide range of frequencies, from one or several cycles per second (e.g. neuronal activity, heart rate) up to one cycle per year (e.g. seasonal rhythms). Although many biological rhythms are related to external environmental cycles, such as the daily light–dark cycle or seasonal changes in day length and temperature, most of them persist in the laboratory, even under highly standardized conditions. Knowledge of biological rhythms and their consideration in experimental design can help to reduce the variability of quantitative measurements in laboratory animals.

Over the past 30 years research has been focused mainly on biological rhythms that are synchronized to the environmental 24-h cycle, and investigators have tried to determine whether these rhythms reflect only a passive reaction to periodic stimulations from the environment (exogenous rhythms) or whether they are an intrinsic property of the organism (endogenous rhythms). Most of the daily fluctuations in behavioural and physiological variables were found to be truly endogenous since they persist even when no external time cues are present. However, without a synchronizing 'zeitgeber' (time giver) the rhythm will slightly deviate from the 24-h period and eventually 'free-run' with an intrinsic natural period. Halberg (1959) introduced the name 'circadian' rhythms (circa diem, L. = about one day) for these endogenous rhythms, and the remaining spectrum of biological rhythms has hence been divided into 'ultradian' rhythms with periods shorter than 24 h and 'infradian' rhythms with periods longer than 24 h (Halberg *et al.*, 1965). Some seasonal rhythms, as well, have been found to persist under constant laboratory conditions. Because their period is approximately 1 year they are classified as 'circannual' rhythms.

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Circadian rhythms

Circadian rhythms are ubiquitous in animals. They have been documented for practically every laboratory species and at every level of organization within an organism, from physiological to biochemical and behavioural processes. In mammals, birds, and several invertebrates investigators have actually been able to find the location of a circadian clock and have discovered some of the underlying physiological mechanisms that generate circadian rhythms.

Functional properties

To investigate the mechanisms generating circadian rhythms one could, in principle, study any behavioural or physiological variable. However, some phenomena have more precise rhythms than others and, thus, are more likely to give reproducible results. Commonly investigated circadian rhythms are those of wheel-running activity or overall locomotor activity of small laboratory animals such as hamsters, rats and mice. Spontaneous locomotor activity not only represents the overall physiological status of an animal (Aschoff, 1962) but it is also a convenient feature to study, because it can be continuously measured for many days using automatic recording equipment with little disturbance to the animal.

Figure 1 shows results of some standard experimental procedures used to determine functional properties of circadian rhythms. It is based on an idealized experiment originally presented by Enright (1981). The data are presented in a 'double plot', a format which is commonly used for recordings of locomotor activity but could also be applied to other behavioural or physiological variables. The first part illustrates a free-running circadian rhythm after elimination of all external time cues. In such an experiment, the animal would be kept in a soundproof chamber under conditions of constant illumination or darkness and constant temperature, with food and water available *ad libitum*. The period of the free-running rhythm (τ) depends on intrinsic factors, such as the physiological status of the individual animal

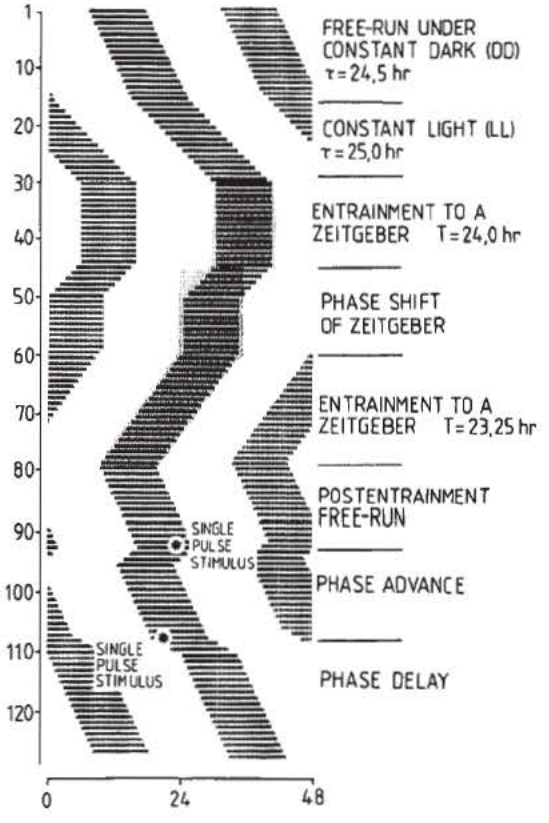

Fig. 1. Idealized data from a hypothetical experiment designed to show period changes of a free-running rhythm caused by internal (age, hormones) or external (light, temperature) stimuli, entrainment by a strong zeitgeber, and phase shifts in response to a single pulse stimulus. The data are presented as a double plot, a format commonly used for the recordings of locomotor activity, where the horizontal axis covers a 48-h period and successive days are plotted from top to bottom. The black horizontal bars represent the physiological variable under investigation, for example wheel-running activity or food intake. A vertical sequence of these bars represents a rhythm with a period of 24 h. A diagonal sequence represents a free-running rhythm with a period longer or shorter than 24 h. The upper portion of the record shows a free-running rhythm with periods of $\tau = 24{\cdot}5$ h and $\tau = 25$ h. Such a lengthening of the free-running period can be observed, for example, after transferring the animal from continuous dark (DD) to constant light conditions (LL) or after changing the hormonal status of the animal. The middle portion shows entrainment to a zeitgeber with a period of $T = 24{\cdot}0$ h (i.e. a light–dark cycle), entrainment to a phase shift of the zeitgeber, and entrainment to a zeitgeber with a period of $T = 23{\cdot}25$ h. The lower portion of the record shows the effect of single-pulse stimuli on the circadian rhythm. The first stimulus caused a phase advance, the second a phase delay. The times at which the phase-shifting stimuli were given are indicated by asterisks. (Adapted from Enright, 1981.)

(e.g. age, hormone levels), and its genetic background (e.g. diurnal or nocturnal species), and on external factors, such as environmental conditions (e.g. light intensity, temperature) and the experimental history of the animal (e.g. breeding conditions, previous experiments) (reviewed in: Aschoff, 1979a,b; Pittendrigh & Daan, 1976a). Nonetheless, the internal clock is remarkably stable (Pittendrigh & Calderola, 1973). With the exception of light intensity, these factors have only small effects on the average free-running period. Changes in light intensity have a dramatic effect on the free-running period. Under constant darkness (DD) τ tends to be longer than 24 h in many diurnal mammals and shorter than 24 h in nocturnal species. With an increase in light intensity under constant light (LL) τ lengthens in both diurnal and nocturnal mammals (Aschoff, 1979a,b).

In the presence of a 24-h environmental cycle, for example a light–dark (LD) regime, the free-running rhythm adjusts to this zeitgeber and exhibits a period of exactly 24 h. This adjustment is called 'synchronization' or 'entrainment' and is represented in the second part of Fig. 1. To verify the entrainment one usually demonstrates that the rhythm follows a phase shift, i.e. a single displacement of the zeitgeber along the time axis. The development of a steady-state (i.e. stable) phase relationship between the external zeitgeber and the internal rhythm usually requires several cycles. After termination of entrainment the free-run must always start at a reproducible phase relative to that seen during prior entrainment (Enright, 1981). The strongest zeitgeber for most animals is the light–dark cycle. Other environmental factors that can entrain circadian rhythms are temperature cycles (Aschoff & Tokura, 1986; Tokura & Aschoff, 1983), food availability (Boulos & Terman, 1980), and social cues, which in primates and humans seem to be as important for the entrainment of circadian rhythms as the light–dark cycle (Wever, 1982).

Although entrainment of the free-running rhythm is not restricted to periods of exactly 24 h, the range of periods to which the internal circadian clock can be entrained by a zeitgeber is limited. In mammals this range is relatively narrow (20–28 h; Aschoff & Pohl, 1978) and depends on the strength (amplitude) of the zeitgeber as well as the strength of the endogenous circadian system of the organism. If the 'range of entrainment' is exceeded, the circadian rhythm will free-run with a period close to that observed under constant conditions. Even then, the signals of the zeitgeber can still modulate the free-running pattern by periodically entraining the rhythm, a phenomenon called 'relative coordination'.

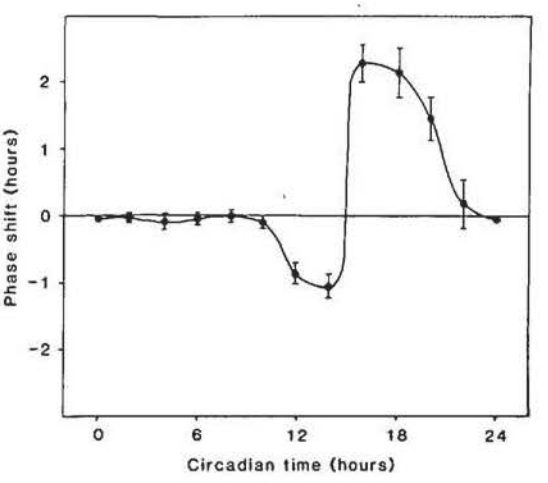

Fig. 2. Phase-response curve (PRC) illustrating the phase-shifting effect of 60-min light pulses on the wheel-running activity rhythm of golden hamsters. The timing of the light pulses is expressed as circadian time (CT) relative to activity onset (= CT 12). Phase advances are plotted as positive phase shifts, phase delays as negative phase shifts. Each point represents the mean phase shift of 6 animals; vertical bars indicate ± SD (from Takahashi & Zatz, 1982; ©Copyright 1982 by the AAAS).

It should be emphasized that measurements of circadian rhythms such as locomotor activity or hormonal profiles do not directly represent the state of the internal pacemaker system, since the pacemaker signal can be subject to many internal and external modifications. The genuine status of the pacemaker system is described only by the period length under free-running conditions and the steady-state phase under entrainment

(Pittendrigh, 1981). In Fig. 1, the last part of the data represents a common experiment used to examine the effects of light or other stimuli, such as hormones, neurotransmitters, and drugs, on the circadian clock. If an animal that has been kept in constant darkness is exposed to a light pulse of 1–60 min, the timing of the activity onset will be permanently modified, so that its free-running rhythm will show a 'phase-shift'. Depending on the time of day at which it is applied the light pulse may induce an advance or a delay of the activity onset or have no effect at all. The time of the light pulse is usually given in 'circadian time' relative to the activity onset. In nocturnal species the activity onset is defined as circadian time 12 (CT 12); the animal is active during its 'subjective night' (CT 12–24) and is inactive during its 'subjective day' (CT 0–12). The magnitude and direction of the phase shift is plotted in a 'phase response curve' (PRC) as a function of the circadian time at which the stimulus was given. PRCs to light pulses have the same general shape as shown in Fig. 2 for both nocturnal and diurnal animals (reviewed in Daan & Pittendrigh, 1976). If the light pulse is given during the late subjective night (CT 18–24) the rhythm will be advanced; if it is given during the early subjective night (CT 12–15) the rhythm will be delayed. If the light pulse is given during the subjective day (CT 0–12), little or no phase shifts occur. The PRC also predicts the repeated daily adjustments necessary to synchronize the endogenous free-running period (τ) to the period (T) of the external light–dark cycle. Consequently, a phase delaying light pulse causes a temporary lengthening of the period, and a phase advancing light pulse causes a temporary shortening of the period. Under conditions of entrainment the daily phase shift $_{\Delta}\phi$ is equal to the difference between the entraining period T and the free-running period τ: $_{\Delta}\phi = T - \tau$ (Daan & Pittendrigh, 1976; Pittendrigh, 1981). The PRC approach has been used in mammals to describe how light or administration of various drugs and neurotransmitters can alter the phase and period of the circadian clock (Turek, 1985, 1987; Zatz, 1979; Zatz & Brownstein, 1979, 1981; Zatz & Herkenham, 1981) thus providing clues as to which neural mechanisms might be involved in the generation and entrainment of circadian rhythms.

Neuronal organization of biological rhythms in mammals

The location of the circadian clock in mammals was first discovered by Richter (1967). After removing various endocrine glands and lesioning specific parts of the rat brain he concluded that a circadian clock must be located near the 'ventral median nucleus' of the hypothalamus. Further studies in the 1970s determined the primary location more specifically in the suprachiasmatic nuclei (SCN) of the hypothalamus (Moore & Eichler, 1972; Stephan & Zucker, 1972a).

The SCN are bilaterally symmetrical nuclei located near the third ventricle and directly above the optic chiasm (Fig. 3a). The SCN of rodents have an oval shape, those of cats and monkeys are more irregularly shaped (Lydic *et al.*, 1982). In rats, each nucleus is about 300 μm in transverse diameter and 600 μm in length and contains about 8000 neurons organized in recognizable subpopulations (Van den Pol, 1980). So far, two specific substances, the peptides vasopressin and vasoactive intestinal polypeptide (VIP), have been identified as being produced by SCN neurons, although they are only found in less than half of all neurons (Sofroniew & Weindl, 1982). Other neuropeptides and neurotransmitters, such as somatostatin, serotonin, corticotropin-releasing factor, avian pancreatic polypeptide, neuropeptide Y, and gamma-aminobutryc acid (GABA) have been found in afferent nerve fibres to the SCN (Card & Moore, 1982; Groos, 1982; Sofroniew & Weindl, 1982; Van den Pol & Tsujimoto, 1985).

The primary input into the SCN is the retinohypothalamic tract (RHT) through which the entraining signals of the external light–dark cycle reach the internal clock (Hendrickson *et al.*, 1972; Moore & Lenn, 1972). The RHT is common to all mammals studied so far. It leaves

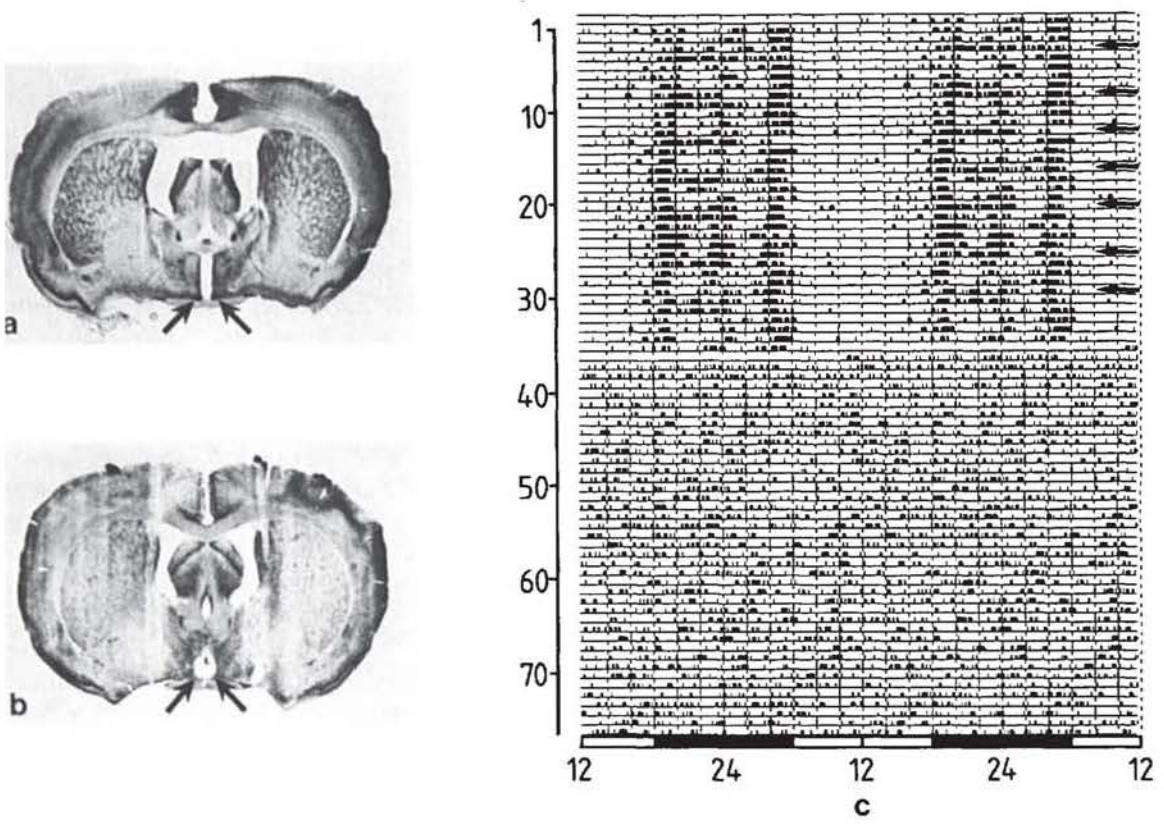

Fig. 3. Effect of a complete SCN lesion on the wheel-running activity pattern of a laboratory rat. (a) Coronal section through the intact midbrain (60 μm thick, thionin staining). The arrows indicate the bilateral SCN. (b) Coronal section as in (a) after complete SCN lesion. The arrows indicate the site of the lesion. (c) Double plotted wheel-running activity record of a female LEW/Ztm rat maintained under a light–dark cycle of 12 h light and 12 h dark. Day time hours and the light–dark schedule are indicated below the record. Numbers to the left denote the day of the experiment. Each raster mark in the record represents a certain number of wheel revolutions over 15 min. The upper portion illustrates the wheel-running activity pattern normally seen in LEW/Ztm females. The days of oestrus are indicated by small arrows on the right hand side of the record; the oestrous cycle was monitored by vaginal smears. Note the oestrous correlated changes in the onset, level and pattern of the wheel-running activity. Rhythmicity disappeared completely after the SCN was lesioned on day 36 of the experiment.

the optic nerve at the optic chiasm and terminates in the SCN (Moore, 1978). In mammals, both blinding and bilateral transection of the optic nerve between retina and optic chiasm totally eliminate entrainment of circadian rhythms to light, while transection of the optic tract beyond the optic chiasm does not disturb entrainment in an obvious manner (Stephan & Zucker, 1972b; Moore & Klein, 1974; Moore, 1978). However, more detailed physiological and anatomical studies have revealed that some information can reach the SCN through other pathways (Groos, 1982; Rusak, 1982; Sofroniew & Weindl, 1982; Moore & Card, 1985). In addition to the direct visual input through the RHT, the SCN receive indirect visual input via the ventral lateral geniculate nuclei (Swanson *et al.*, 1974; Card & Moore, 1982; Harrington *et al.*, 1987; Pickard, Ralph & Menaker, 1987), which are part of the central visual system. Another indirect visual input might reach the SCN through serotonergic fibres from the raphe nuclei (Ajika & Ochi, 1978) which themselves receive a retinal input (Foote *et al.*, 1978). Neural inputs from the septum,

hypothalamus, thalamus, hippocampus and midbrain as well as commissural connections between the bilateral SCN have also been demonstrated (Pickard, 1982). Efferent projections of the SCN terminate in other hypothalamic nuclei (paraventricular nucleus, dorsomedial hypothalamic nucleus), in the thalamus (paraventricular thalamic nucleus) and in the midbrain (Swanson & Cowan, 1975; Berk & Finkelstein, 1981; Stephan *et al.*, 1981; Sofroniew & Weindl, 1982).

The following experiments indicate that the SCN play an important role in the normal expression of circadian rhythms:

(1) SCN lesions: A variety of behavioural and physiological circadian rhythms, including locomotor activity, feeding, drinking, body temperature, synthesis and/or secretion of several hormones, and the sleep–wake cycle, can be severely disrupted or completely abolished by complete bilateral SCN lesions or isolation of the SCN from other neural structures (reviewed in: Moore, 1978, 1983; Rosenwasser & Adler, 1986; Rusak & Zucker, 1979; Turek, 1985). An example is given in Fig. 3c which illustrates the effect of complete SCN lesions on the wheel-running activity pattern of laboratory rats. Follow-up studies on rats whose SCN had been lesioned immediately after birth have shown that the function of the SCN were not taken over by other parts of the brain (Mosko & Moore, 1978). SCN lesions also disrupt oestrous cyclicity and photoperiodic time measurement, two components of the reproductive process that depend upon the circadian system for temporal information (Rusak & Morin, 1965; Stetson & Watson-Whitmyre, 1976; Turek *et al.*, 1980).

(2) Demonstration of intrinsic oscillations: Circadian rhythms in multi-unit neuronal activity have been recorded in the SCN and several other regions of the rat brain. After surgical isolation of the SCN within a 'hypothalamic island', circadian firing patterns persist in the SCN but can no longer be recorded in other parts of the brain (Inouye & Kawamura, 1979). This indicates that circadian rhythms in neuronal activity originate in the SCN. The neural circadian rhythms in the SCN showed characteristic properties (e.g. a free-running period) expected of an endogenous circadian oscillator (Inouye & Kawamura, 1982; Inouye, 1984). The SCN seem to maintain a neural or hormonal circadian rhythm even *in vitro* (Green & Gillette, 1982; Shibata *et al.*, 1984; Earnest & Sladek, 1987). Using 2-deoxyglucose autoradiography, researchers have also found that in a wide range of species glucose consumption follows a circadian rhythm in the SCN (Flood & Gibbs, 1982; Schwartz, Reppert, Eagan & Moore-Ede, 1983). SCN metabolic activity is high during the subjective day and low during the subjective night in both nocturnal and diurnal animals (Schwartz *et al.*, 1983). In rats the circadian rhythm of glucose uptake in the SCN can be detected as early as several days before birth, prior to the arrival of any retinal projections (Reppert & Schwartz, 1983, 1984).

(3) Electrical and chemical stimulations: In hamsters and rats, short electrical stimulation of the SCN has a similar phase-shifting effect on the circadian rhythm as light (Rusak & Groos, 1982). Timed injections of pharmacological agonists or antagonists of neurotransmitters found in SCN cells and axons have been shown to mimic or block the phase-shifting effect of light (reviewed in: Turek, 1985, 1987; Rosenwasser & Adler, 1986). For example, photic stimulation increases the concentration of acetylcholine in the SCN (Murakami *et al.* 1984).

Intracerebroventricular injections of carbachol, an acetylcholine agonist, mimic the phase shifting effect of light (Zatz & Herkenham, 1981; Earnest & Turek, 1983), whereas mecamylamine, a cholinergic antagonist, blocks the phase-shifting effect of light (Keefe *et al.*, 1987). This suggests that acetylcholine may be involved in the transmission of light–dark information to and within the circadian clock.

(4) SCN-Transplantation: Only recently has the pacemaker function of the SCN been demonstrated in the most direct way. By transplanting fetal neural tissue containing the anlagen for the SCN into arrhythmic SCN-lesioned hamsters and rats circadian rhythmicity of locomotor activity has been restored (Kawamura & Nithonmatsu, 1985; Aguilar-Roblero *et al.*, 1986; Lehman *et al.*, 1987). It remains to be shown whether pacemaker properties such as phase and period of the donor can also be transferred. This would demonstrate that the transplanted tissue actually generates the restored rhythm rather than simply having a permissive effect. The specificity of transplanting SCN tissue has been questioned lately after it was observed that circadian rhythms could also be restored by transplanting fetal cortical tissue into the lesion site (García-Hernández *et al.*, 1987).

The circadian system of mammals is thought to consist of multiple circadian oscillators which are coupled to each other in both a hierarchical and non-hierarchical (mutual) manner (reviewed in: Pittendrigh, 1960, 1974; Rusak, 1979; Rosenwasser & Adler, 1986). While these oscillators normally are synchronized in order to generate a single pacemaker signal, certain conditions can cause their desynchronization.

The most prominent example of desynchronization is 'splitting', the dissociation of the circadian rhythm into two or sometimes even more distinct components (reviewed in: Pittendrigh, 1974; Pittendrigh & Daan, 1976b; Turek *et al.*, 1982; Rosenwasser & Adler, 1986). A characteristic feature of splitting is the temporary free-run of the two components with different periods. The two components often resynchronize after reaching a 180° phase-relation and subsequently free-run with identical periods. Splitting has been reported for a number of mammalian species, including hamsters (Earnest & Turek, 1982; Turek *et al.*, 1982), rats (Boulos & Terman, 1979; Cheung & McCormack, 1983), tree shrews (Hoffman, 1971) and monkeys (Fuller *et al.*, 1979). In hamsters it has been evoked by exposing the animals to long-term bright light conditions or SCN lesions (Rusak, 1977; Earnest & Turek, 1982). In other species splitting has also been observed after long-term exposure to constant dark (Hoffman, 1971), administration of pharmacological substances (Wirz-Justice, Groos & Wehr, 1982), and manipulations of the endocrine system by gonadectomy and hormone replacement (Morin, 1980; Morin & Cummings, 1982). In hamsters, splitting disappears rapidly, when the animals are transferred into constant darkness (Earnest & Turek, 1982). The splitting phenomenon has led to the assumption that at least two mutually coupled oscillators underlie the circadian locomotor activity rhythm (Pittendrigh & Daan, 1976b). Because of the splitting phenomenon and the fact that even the undisturbed, non-split activity rhythms of many mammalian species display quite complex patterns made up of two or more activity components (Aschoff, 1962), it is widely accepted today that the circadian clock of mammals is a multi-oscillatory system.

Although multiple circadian oscillators are assumed to lie in the SCN, there is also evidence for the presence of oscillators outside the SCN. For example, not all circadian rhythms disappear after complete SCN lesions. In hamsters, both circadian and ultradian rhythms in wheel-running activity have been reported to persist in individual animals (Rusak, 1977). In rats, periodic feeding can entrain food-anticipatory rhythms in a number of physiological parameters (reviewed in: Boulos & Terman, 1980; Aschoff *et al.*, 1983), and those rhythms persist after SCN lesions showing the same characteristics as before (Krieger *et al.*, 1977; Stephan *et al.*, 1979a,b; Stephan, 1982, 1984). These results indicate that the food entrainable pacemaker is functionally and anatomically independent from the 'classical', i.e. light entrainable, circadian system within the SCN (Gibbs, 1979; Boulos *et al.*, 1980). The search for the food entrainable pacemaker has focused on the ventromedial nucleus of the hypothalamus because destruction of this nucleus can abolish the food-anticipatory rhythms (Krieger, 1980; Inouye, 1982). Recently,

the circadian locomotor activity rhythm of completely arrhythmic SCN lesioned rats could be restored by chronic administration of methamphetamine in the drinking water (Honma *et al.*, 1987). Since the rhythm was obviously not generated by the SCN nor entrained by the light–dark cycle, one must assume that the methamphetamine activated an endogenous oscillatory mechanism outside the SCN that is probably identical with the food-anticipatory pacemaker system.

Genetic determination

A number of characteristic properties of circadian rhythms have been shown to be genetically determined. To investigate the genetic background of circadian rhythms in mammals quantitative genetic methods of variance and covariance analysis (Hegmann & Possidente, 1981) have been applied to inbred strains of small laboratory animals like mice, hamsters and rats (Table 1). In mice, there are significant differences between strains in the free-running period of wheel-running activity (Ebihara *et al.*, 1978) and food and water intake (Possidente & Hegmann, 1980) as well as in the circadian phase and period of body temperature (Connolly & Lynch, 1981, 1983). Furthermore, a positive genetic correlation was found between the phase and the period of the circadian rhythms of food intake and water consumption, which suggests that both parameters are regulated by a common physiological mechanism and would respond to natural selection as a single circadian complex under common gene control (Possidente & Hegmann, 1980). In hamsters, strain differences have been demonstrated for the free-running period, the circadian phase of activity onset and splitting (Hotz & Turek, 1987). Recently, a spontaneous hamster mutant has been isolated that has a remarkably short free-running period of only 20 h (Ralph & Menaker, 1987). In rats, strain differences have been demonstrated for the free-running period and the daily pattern of spontaneous locomotor activity (Büttner & Wollnik, 1984). Two inbred strains of rats, BH/Ztm and LEW/Ztm, showed an unusual bimodal and trimodal, respectively, activity pattern. A classical genetic analysis revealed that the trimodal activity pattern of the LEW/Ztm strain has a recessive single-gene mode of inheritance (Wollnik *et al.*, 1987).

Table 1. Inbred strains of laboratory mammals used for genetic analyses of biological rhythms

Mice	BALB/CBYJ	Possidente & Hegmann (1980)
	BALB/cIbg	Connolly & Lynch (1981)
	C3H/HeJ	Possidente & Hegmann (1980)
	C3H/2Ibg	Connolly & Lynch (1981, 1983)
	C57BL/6J	Connolly & Lynch (1981, 1983)
		Ebihara *et al.* (1978)
	C57BL/10Sn	Ebihara *et al.* (1978)
		Possidente & Hegmann (1980)
	C58/J	Possidente & Hegmann (1980)
	DBA/1BG	Connolly & Lynch (1981)
	DBA/1J	Possidente & Hegmann (1980)
	DDK	Ebihara *et al.* (1978)
	MOL-A	Ebihara *et al.* (1978)
Rats	ACI/Ztm	Büttner & Wollnik (1984)
	LEW/Ztm	Wollnik *et al.* (1987)
	AS/Ztm	Büttner & Wollnik (1984)
	BH/Ztm	
	BS/Ztm	
Golden hamsters	CB	Hotz & Turek (1987)
	LHC	
	LSH	
	MHA	
	PD4	

Another approach to investigate the genetic background of circadian rhythms is to study strains with known mutations in the visual or central nervous system. Mutations which in early development modify retinal pigment and visual pathways also modify developmental and functional properties of circadian rhythms. For example, the free-running circadian period of both albino and pinkeye-dilute mice is shorter than that of pigmented mice (Possidente *et al.*, 1982). Mice of an anophthalmic strain (ZRDCT-An) with varying degrees of hypogenesis of the mediobasal hypothalamus and SCN that showed a partially or completely arrhythmic wheel-running activity pattern had less than one third of the typical number of cells in the SCN (Scheuch *et al.*, 1982). A strain of laboratory rats with a genetic inability to produce vasopressin (Long Evans, Brattleboro strain) showed no obvious alterations or deficits in their circadian

rhymicity (Peterson *et al.*, 1980) indicating that the circadian clock does not involve the vasopressin neurons of the SCN. Inbred strains of laboratory animals with genetically fixed modifications of the circadian system would provide a good test case for SCN transplantation experiments, since the transfer of characteristic properties of a clock mutant could easily be verified.

Ultradian rhythms

Ultradian rhythms are defined as biological rhythms with a period significantly shorter than 24 h, ranging from 1 to 12 h (Halberg *et al.*, 1965). Typical examples of ultradian rhythms are the repetition of rapid eye movements (REM) every 90 min during sleep in humans and some mammalian species and ultradian rhythms in physiological parameters associated with REM sleep (reviewed in Schulz & Lavie, 1985). Other examples are episodic secretion patterns of various hormones, such as luteinizing hormone (peaks every 0·5–3 h), growth hormone (peaks every 3 h), and corticosterone (reviewed in Van Cauter & Honinckx, 1985), and short-term behavioural rhythms in locomotor activity or food intake of small mammals (Gerkema & Daan, 1985; Honma & Honma, 1985a). Ultradian rhythms are more difficult to investigate than circadian rhythms for the following reasons. First, ultradian rhythms include a wide range of rhythmic phenomena which do not necessarily share common properties. For example, some ultradian rhythms are true periodic processes with a consistent period, whereas others are rather episodic with variable time lags between single events (Aschoff & Gerkema, 1985). Second, unlike circadian rhythms, which have evolved in adaptation to the 24-h fluctuations of the environment, ultradian rhythms do not correspond to any known physical cycle in the environment, and a general functional significant like that of circadian rhythms has not been demonstrated yet (Gerkema & Daan, 1985). Third, in order to detect ultradian rhythms in raw data and to discriminate truly rhythmic phenomena from random stochastic processes it is often necessary to use elaborate recording and analysing techniques, such as periodogram and power spectrum analyses (Van Cauter, 1981; Wollnik & Döhler, 1986).

Since many ultradian rhythms are related to metabolic processes (e.g. food intake, hormone secretion, urinary excretion) and sometimes even show an allometric correlation between period and body weight (Daan & Slopsema, 1978; Gerkema & Daan, 1985), several authors have suggested that digestive functions are involved in the generation of ultradian rhythms (reviewed in: Aschoff, 1962; Daan & Aschoff, 1981). However, it is now well established that their most likely site of generation is the nervous system (reviewed in Schulz & Lavie, 1985). Two general models for the generation of ultradian rhythms have been proposed. The first assumes the existence of an ultradian pacemaker system that is anatomically and functionally independent from the circadian system. It was developed by Kleitman (1961, 1982) and is based on data on the 90-min sleep–wake cycle in humans. This independent ultradian pacemaker system can either be a periodic oscillatory system or a homeostatic renewal process (Gerkema & Daan, 1985). The second model views ultradian rhythms as a result of desynchronization of several circadian oscillators that are coupled to each other in different phase relations (Pittendrigh, 1974). Considering the broad variety of ultradian rhythms it is not surprising that results have been reported in support of both models.

For example, an independent ultradian oscillator appears to generate the feeding and locomotor activity rhythms in the common vole (Daan & Slopsema, 1978; Daan & Aschoff, 1981). In this species a total lesion of the SCN completely abolished the circadian rhythm but did not affect the ultradian rhythmicity (Gerkema & Daan, 1985). Similarly, complete SCN lesions in rats did not disrupt the pulsatile pattern of LH release (Soper & Weick, 1980) suggesting that ultradian and circadian rhythms in hormone release are generated by different neural centres. In both cases, investigators identified the arcuate nucleus (ARC) of the hypothalamus as a possible

candidate for the ultradian pacemaker system (Gerkema & Daan, 1985; Knobil & Hotchkiss, 1985). The same neuronal site has also been suggested as the location of a pacemaker for ultradian rhythms in growth hormone release in rats (Eikelboom & Tannenbaum, 1983). However, even if ultradian rhythms are generated by an independent ultradian pacemaker they can still be modulated by the circadian system. Such a modifying influence of the circadian system has been found in the pulsatile patterns of hormone release (reviewed in Turek & Van Cauter, 1988) and the behavioural ultradian rhythms of voles (Gerkema & Daan, 1985).

Behavioural activity patterns of small laboratory mammals that show short-term fluctuations are also often referred to as ultradian rhythms (reviewed in: Aschoff, 1962; Daan & Aschoff, 1981). In laboratory rats, behavioural ultradian rhythms appear prior to circadian rhythms during early developmental stages (Honma & Honma, 1985b). They are also prominent during aging (Albers *et al.*, 1981a) and under long-term exposure to constant light (Albers *et al.*, 1981a; Honma & Honma, 1985a) when the circadian rhythmicity fades or disappears. These findings have even led to the suggestion that ultradian rhythms may be the underlying time pattern from which circadian rhythms are then derived by frequency and amplitude modulation (Honma & Honma, 1985a,b).

The following observations on behavioural ultradian rhythms in laboratory rats, however, do not support the existence of an independent ultradian pacemaker. First, ultradian activity rhythms in adult laboratory rats have so far been described only for particular animals (Stephan, 1983) or specific inbred strains, e.g. LEW/Ztm (Büttner & Wollnik, 1984), and may thus not be characteristic for the normal temporal organization of rat behaviour. Second, since complete SCN lesions in the inbred strain LEW/Ztm abolished both ultradian and circadian rhythms of wheel-running activity, the SCN is apparently involved in the control of both rhythms (Wollnik & Turek, 1987). Third, the expression of ultradian rhythms in the adult LEW/Ztm rats depends on the hormonal status of the animals; high serum levels of oestrogen suppress ultradian rhythms in both male and female rats (Wollnik & Döhler, 1986). Since similar effects of hormones on the coupling between circadian oscillators have been demonstrated for hamsters (Morin, 1980) and mice (Daan *et al.*, 1975), ultradian rhythms in locomotor activity may be the result of the combination of several circadian oscillators coupled to each other in various phase relations.

The diversity of ultradian rhythms is also apparent in the domain of their functional relevance. For example, the normal reproductive function of humans and primates depends on a specific ultradian timing of the release of gonadotropin-releasing hormone by the hypothalamus and luteinizing hormone by the pituitary (Knobil & Hotchkiss, 1985). In voles, the ultradian feeding and activity rhythms are essential for providing optimal timing of food intake and digestive pauses as well as interindividual social synchrony among conspecifics in order to reduce the predator risk for the individual animal (Daan & Slopsema, 1978; Daan & Aschoff, 1981).

Ultradian rhythms often have periods of 3, 4, 4·8, or 6 h. Since these periods are subharmonics of the circadian period, one must verify that they are clearly detectable in the raw data and not mere artifacts of the statistical method used for data analysis. The predominance of integer ratios of the circadian period can be interpreted both as a result of the desynchronization of a multi-oscillatory circadian pacemaker system and as a sign for independent ultradian oscillators that have evolved during the evolutionary process because they could maintain a constant phase relationship with the circadian rhythm (Broughton, 1985). Organisms with ultradian rhythms of 3, 4, 4·8 or 6 h periods should have significant advantages over organisms with ultradian rhythms of, for example, 1·7, 5 or 7 h periods, since the latter would never stay in phase with each other and the major environmental periodicities.

Although there is no doubt today that many behavioural and physiological events follow an ultradian temporal pattern, no other class of biological rhythms is characterized by such a diversity of rhythmic phenomena, generating mechanisms, and biological functions. Therefore, caution is warranted when results from observations of one ultradian phenomenon are being generalized to other ultradian rhythms.

Infradian rhythms

Infradian rhythms are defined as biological rhythms with a period significantly longer than 1 day, but less than 1 year (Halbert *et al.*, 1965). In mammals, many infradian rhythms are related to functional changes in the ovary and can therefore be observed only in females of spontaneous ovulatory species. Oestrous is induced by changes in the levels of the pituitary hormones luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (reviewed in Mahesh, 1985) and occurs at regular intervals characteristic for each species (mice, rats, hamsters: once every 4–5 days, guineapigs, sheep, goats: once every 13–20 days). The resulting physiological changes in the ovaries include fluctuations of oestrogen and progesterone secretion which in turn influence behavioural parameters such as spontaneous locomotor activity, food intake, aggression and sexual behaviour (reviewed in Campbell & Turek, 1981).

The period of behavioural heat on or around the day of ovulation is characterized by an increased level of activity (rats: Albers *et al.*, 1981b, mice: Guttman *et al.*, 1975, hamsters: Finkelstein *et al.*, 1978). In hamsters and rats, activity starts earlier on the day of oestrous resulting in a 'scalloping' of the activity onset in intervals of 4 or 5 days (Albers *et al.*, 1981b; Morin *et al.*, 1977; Sridaran & McCormack, 1977). Furthermore, the activity pattern undergoes complex changes during the oestrous cycle (Takahashi & Menaker, 1980). Figure 3c shows an example of oestrous-correlated modulation of wheel-running activity in a laboratory rat. On the day of ovulation activity starts earlier and retains a constant high level throughout the activity phase. On the days following ovulation, activity starts later and shows a bi- or trimodal pattern. By looking at continuous recordings of locomotor activity one can easily predict the day of ovulation. Changes in the onset, intensity and pattern of wheel-running activity are almost as reliable a marker of the oestrous cycle as vaginal smears (Sridaran & McCormack, 1977).

The oestrous cycle is an endogenous rhythm that depends on various components of the neuroendocrine system located in the ventromedial arcuate region and the preoptic suprachiasmatic area of the hypothalamus (reviewed in Mahesh, 1985). A striking feature of the oestrous cycle is the circadian timing of many oestrous related events. For example, ovulation and the LH surge on the day of proestrus maintain a constant phase relation with the external light–dark cycle and follow a phase shift of this zeitgeber (Alleva *et al.*, 1968; Stetson & Gibson, 1977; Gallo, 1981). Even in the absence of external time cues, the oestrous cycle is closely associated with the circadian system. For example, the oestrous cycle of hamsters and rats free-runs with a period 4 or (in some rats) 5 times that of the circadian activity rhythm (Fitzgerald & Zucker, 1976; Albers *et al.*, 1981b). Female hamsters whose activity rhythm was split after exposure to constant light show two LH surges, each occurring 0–4 h before the onset of one activity component (Swann & Turek, 1985).

The circadian timing of the oestrous cycle seems to be controlled by the SCN. Bilateral lesions of the SCN not only abolish the preovulatory LH surge and thus impede ovulation but also cause a state of persistent oestrous in hamsters and rats (Stetson & Watson-Whitmyre, 1976). Since the preovulatory LH surge is normally triggered by an increased secretion of gonadotropin releasing hormone (GnRH) from the mediobasal hypothalamus (Levine & Ramirez, 1982) the SCN must somehow be coupled to those GnRH neurons. However, it is not clear yet which pathways and neurotransmitters are involved in the transmission of the circadian timing signal to the mediobasal hypothalamus (reviewed in: Turek & Van Cauter, 1988).

Except those infradian rhythms associated with the oestrous cycle only few others have been reported. For example, in dormice kept under various laboratory conditions infradian rhythms with a period of about 2 months have been demonstrated for several physiological functions, such as body weight, food and water intake, and weight of liver, testis, and adrenal and salvary gland (Mrosovsky *et al.*, 1980). Another example is the weekly, or circaseptadian, rhythm of enzyme activity in the rat pineal (Vollrath, 1982). Since this weekly rhythm was only found in animals that had been kept under normal laboratory conditions and had not been demonstrated to free-run with a period of about 7 days, it is likely that it is an example of an exogenous rhythm induced by periodic events in the laboratory environment, such as work schedules of animal care personnel, heater operation, street noise, etc.

Seasonal and circannual rhythms

Many biological activities and reproductive functions are restricted by environmental conditions such as temperature, day length, or food availability to a time of the year when they are most likely to be successful. In addition, many behavioural, physiological and morphological processes undergo annual fluctuations that are directly or indirectly related to seasonal variations in the environment (reviewed in Gwinner, 1981). These seasonal rhythms may be referred to as 'circannual' rhythms only if it is has been demonstrated that they persist with a period of about 1 year for at least two cycles under constant environmental conditions. Annual rhythms of body weight or reproductive functions that can be observed in many mammals under natural conditions have been confirmed as a circannual rhythm only in a few species, such as ground squirrels (Pengelly & Asmundson, 1974).

In many seasonal breeding animals, seasonal changes in reproductive functions are not observed under constant laboratory conditions. Their reproductive state is a direct result of the day length, or 'photoperiod', and thus can be manipulated using different light–dark cycles (reviewed in: Hoffmann, 1981; Keefe & Turek, 1985). Much of our understanding of the neuroendocrine events involved in the photoperiodic control of reproduction is derived from studies on three mammalian species: the golden hamster and the Djungarian hamster (both long-day breeders) and the sheep (a short-day breeder). In sheep, long photoperiods (more than 13 h light per day) induce reproductive quiescence, whereas short photoperiods (less than 12 h light per day) stimulate gonadal reactivation and reproduction; in hamsters the effect is just the opposite. However, the photoperiod induced changes are not permanent. In hamsters, for examples, the short-day induced gonadal quiescence persists only for about 18 weeks. After 18 weeks gonadal reactivation may occur even if the animal remains exposed to short days (Turek *et al.*, 1975). Before gonadal quiescence can be induced by short photoperiods again, the hamsters would have to be kept under long day conditions for several weeks (Stetson *et al.*, 1977).

Photoperiodic animals can measure the length of day very precisely. In early work on the mechanisms of photoperiodic time measurement it was assumed that animals determine the total duration of light and darkness by some sort of 'hourglass' mechanism or interval timer (Farner *et al.*, 1953). Since then several experiments using unusual light–dark cycles ('resonance', 'T' and 'night interrupt' experiments) have revealed, that animals measure the length of day by comparing the external light–dark cycle against an endogenous circadian rhythm of light sensitivity (reviewed in Keefe & Turek, 1985). In those experiments light was presented only for short periods under otherwise constant darkness. The animal interpreted as either long or short days only those light pulses that were applied during a certain phase of the circadian photosensitivity rhythm.

An important part of the photoperiodic time measurement seems to be the circadian system in the SCN. Bilateral lesions of the SCN abolish photoperiod induced changes in the reproductive status; lesioned animals retain their gonadal

function regardless of the light–dark cycle (hamsters: Rusak & Morin, 1976; Stetson & Watson-Whitmyre, 1976; ewes: Domanski *et al.*, 1980). The neural information about day length is transferred to the endocrine hypothalamic-pituitary-gonadal axis via the pineal organ (Reiter, 1980a,b). Using anatomical and physiological techniques the following pathway between the SCN and the pineal organ has been discovered (reviewed in Keefe & Turek, 1985). SCN efferents project to regions of the paraventricular nuclei of the hypothalamus (Klein *et al.*, 1983; Pickard & Turek, 1983; Lehman *et al.*, 1984; Inouye & Turek, 1986). The paraventricular nuclei project to the intermediolateral nuclei of the spinal cord (Swanson & Kuypers, 1980) which innervate the superior cervical ganglia (Rando *et al.*, 1981). A beta-adrenergic input from the superior cervical ganglia controls the activity of pineal N-acetyltransferase, thereby regulating the melatonin synthesis in the pineal organ (Zatz, 1981; Klein, 1985). In a number of photoperiodic species, pinealectomy prevents the short-day induced gonadal regression (reviewed in Hoffman, 1981).

The SCN regulates the rhythmic timing of production of melatonin so that melatonin levels are high at night and low during the day (Goldman, 1983; Goldman & Darrow, 1983). Recent studies on both Djungarian hamsters and sheep suggest that the duration of high night-time levels in circulating melatonin determines whether the photoperiod will be interpreted as a long or a short day (Carter & Goldman, 1983a,b; Bittman & Karsch, 1984). It is not known yet how melatonin alters hypothalamic-pituitary functions and how the signal of melatonin duration is transformed into a change in neuroendocrine gonadal activity.

Conclusion

Biological rhythms have been observed in practically all groups of laboratory animals and at every level of physiological and behavioural organization even under standardized laboratory conditions. Biological rhythms are important for the health and wellbeing of the organism for two reasons. First, they allow an optimal timing of biological activities within the context of physical environmental cycles. Circadian and circannual rhythms serve as biological clocks that enable the organism to anticipate periodic events in its environment so that it can initiate slow processes on time. Second, biological rhythms provide an internal framework for the temporal organization of physiology and behaviour thus contributing to the maintenance of a balanced internal milieu.

Biological rhythms are classified according to their period as circadian, ultradian, infradian, and seasonal or circannual rhythms. In recent years, researchers have discovered some of the underlying pacemaker systems and physiological mechanisms involved in the generation, entrainment and coupling of biological rhythms. Circadian rhythms are generated by a multi-oscillator system located in the SCN and are synchronized to the external 24 h light–dark cycle. Ultradian rhythms can be generated by a variety of different neural pacemaker systems and are characterized by the diversity of their rhythmic phenomena. Many infradian rhythms are the result of changes in gonadal hormone levels during the oestrous cycle and can only be observed in female animals. Many annual rhythms are correlated with changes in the reproductive functions over the year. Photoperiodic changes of reproductive functions are controlled through complex interactions between the SCN, the pineal, and the hypothalamic-pituitary-gonadal axis. Frequency and amplitude of ultradian rhythms are often modulated on a circadian time scale. The circadian system must be considered the most important biological clock since it affects the regulation of all other biological rhythms. Many key events within the oestrous cycle are timed by the circadian system, and the photoperiodic control of seasonal rhythms involves the circadian clock for measuring day length.

Most, if not all, physiological systems are affected by biological rhythms, and laboratory animal science should take this into account in designing experimental schedules. For example,

contradictory results from different laboratories might be due simply to differences in the daily schedules in which experiments are being performed. Different findings on the periodicity of ultradian rhythms, such as episodic hormonal fluctuations, might be due to different sampling intervals. Such pitfalls can be avoided by scanning the unknown rhythmicity of the investigated variable with repeated experiments performed at several time points in the cycle of a rhythm in order to determine characteristic phase points, such as the daily peak or low of a circadian rhythm. These should serve as reference points when selecting the times to perform the real experiments. If this chronobiological approach cannot be taken because of methodological or other constraints, experimenters should at least provide information about their experimental time schedule and the light–dark cycle of their animals in order to allow others to reproduce or compare their results.

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