Circadian Physiology

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Abstract

Circadian physiology refers to biological processes that are rhythmic with the time scale of a day. Parameters of circadian rhythmicity are mean level, phase, period, amplitude, waveform, and robustness. Circadian physiology is an overall feature of life and is well conserved during evolution from unicellular to multicellular organisms. Circadian physiology is governed by a circadian system that is capable to generate – without any environmental cue – self-sustained "free-running" or endogenous rhythms. These endogenous rhythms have a period length of approximately, but not precisely, 24 h; they are thus called circadian rhythms. The circadian system influences a variety of behavioral and autonomic functions. In normal life, circadian rhythms are entrained to (synchronized with) environmental cycles. Environmental cues that are able to synchronize circadian rhythms are called "zeitgebers." For all living organisms, the change between day and night is the most important "zeitgeber."

In multicellular organisms, the circadian system has a hierarchical architecture and comprises a "central" circadian rhythm generator (a "master clock") as well as multiple subsidiary clocks in the periphery (slave oscillators or peripheral clocks). Both the central circadian rhythm generator and the slave oscillators contain a molecular clockwork comprising clock genes and their protein products that interact with each other in transcriptional/translational feedback loops and control the expression of clock-controlled genes. However, there is an important difference between the central circadian rhythm generator, the master clock, and the slave oscillators: while the master clock is capable to generate a self-sustained rhythm, the rhythms generated by the peripheral clocks are lost after several cycles because of desynchronization. To maintain proper phase relationships with each other, the slave oscillators in peripheral organs need to be coordinated by the central rhythm generator. This coordination is achieved by multiple direct and indirect output pathways of the central rhythm generator. According to current concepts, synchronization of peripheral clocks cannot be accomplished by a single "entrainment" signal, but requires a combination (cocktail) of signals. These cocktails appear to differ from one peripheral clock to the other. Moreover, peripheral clocks can be directly entrained by various external (environmental) stimuli.

One of the most complex circadian systems is present in mammals; it drives the rhythm in locomotor activity, influences the sleep-wake cycle, and is involved in the control of body temperature, food intake, and metabolism. Moreover, the circadian system may affect the cell cycle. The central circadian rhythm generator of mammals is located in a distinct region of the brain, the bilaterally arranged suprachiasmatic nuclei (SCN) of the hypothalamus. Circadian rhythm generation

in the SCN involves a molecular clockwork. The circadian rhythm generator in the SCN is entrained to the daily light/dark cycle. Light stimuli, acting as zeitgeber, are perceived by classical and novel photoreceptors in the retina and transmitted to the SCN via the retinohypothalamic tract (RHT), a distinct portion of the optic nerve. The RHT provides an essential input pathway to the SCN. Output pathways of the circadian rhythm generator in the SCN employ neuronal, neuroendocrine, and hormonal mechanisms. The final neuronal output pathway of the SCN is provided by the autonomic (sympathetic and parasympathetic) nervous system. An important neuroendocrine hand of the circadian system is melatonin which is produced night by night in the pineal gland under the control of the SCN. Melatonin represents a chronobiotic; it acts upon specific receptors, feeds back to the SCN, and modulates several autonomic functions. In addition to melatonin, glucocorticoids secreted from the cortex of the adrenal gland represent an important output signal of the circadian system. Glucocorticoids act on glucocorticoid receptors which are widely distributed in the body. Via these rather direct output pathways, the SCN sends timing signals to slave oscillators present in a variety of brain regions outside the SCN and in peripheral organs. SCN-derived timing signals are needed to maintain proper phase relationships among the multiple peripheral clocks, and the phase coherence in peripheral clocks is lost in SCN-lesioned animals. The SCN also provides indirect cues to the clocks in the periphery via its impact on the body temperature rhythm and the rest-activity rhythm which in turn drives the feeding rhythms. The feeding-fasting cycles which under normal conditions are in phase with the rest-activity rhythms appear to be dominant synchronizing signals for the peripheral clocks in the liver, kidney, pancreas, and heart. In these organs, the expression profiles of many circadian genes are influenced by the timing of food intake. This entrainment may be mediated by hormones secreted upon feeding or fasting, e.g., cholecystokinin, ghrelin, or leptin; by food metabolites, e.g., glucose, cholesterol, and fatty acids; by postprandial temperature elevations; and by the intracellular redox state ratio. The molecular clockwork in peripheral tissues controls circadian rhythms in metabolic and physiologic cell/organ function. The importance of molecular clocks is underlined by microarray studies showing that up to 20 % of the genes expressed in peripheral organs (e.g., liver, muscle, adipose tissue) are rhythmic, suggesting that a considerable portion of the transcriptome is controlled by the circadian system. The rhythmically expressed genes encode proteins and enzymes involved in biosynthetic and metabolic processes such as lipid metabolism, glycolysis and gluconeogenesis, oxidative phosphorylation, and detoxification pathways. Notably, in many of these pathways, the rate-limiting enzymes are under circadian control. These data indicate a close interrelationship between the circadian system and energy metabolism.

The circadian system may become altered or disrupted under various conditions. The most frequent reason for alteration of the circadian system in healthy people is the so-called jet lag occurring after rapid travel across a number of time zones. Moreover, disruptions of the circadian system are observed in numerous diseases. These include the familial advanced sleep phase syndrome, the delayed

sleep phase syndrome, seasonal affective disorder, uni- and bipolar depression, autism spectrum disorders, Alzheimer's disease, Parkinson's disease, and Huntington's disease. Sleep disturbances in aging people can be partially attributed to age-related reduction in amplitude and phase advance of circadian rhythms. Finally, the close relationship between circadian and metabolic cycles suggests that the metabolic syndrome may be associated with disturbances of the circadian system.

Keywords

Arcuate nucleus (Arc) • Arylalkylamine N-acetyltransferase (AANAT) • Chronobiology • Circadian physiology • Circadian rhythm • Circadian rhythm generator • Circadian system • Glucocorticoids • Intrinsically photosensitive retinal ganglion cells (ipRGCs) • Melatonin • Molecular clocks, peripheral organs • Molecular clockwork • Molecular clockwork, cell cycle • Peripheral clocks • Restricted feeding • Sleep-wake cycle, circadian system

Brief History

Studies on circadian physiology date back to the eighteenth century when the French astronomer Jean Jacques d'Ortous de Mairan reported in 1729 that the leaves of Mimosa (pea, legumen) open and close rhythmically at day and night and that this rhythm persisted when the plants were deprived from daylight and kept in a dark corner under his desk. Later on, day/night rhythms were observed in many plant and animal species, and these observations supported the concept that such rhythms do not reflect a passive response to the environmental day-night changes, but an endogenous rhythm that originates in the organisms themselves and is adapted to rhythmic environmental changes, such as the astrophysical day.

Modern scientific investigations on circadian physiology were initiated in the twentieth century. Most interestingly, the field has benefitted from a remarkable interdisciplinary and comparative approach which showed the presence of daily rhythms in virtually all living creatures – from unicellular organisms to plants to animals and man. Pioneers in the field of circadian functions were the botanist Erwin Bünning (1906–1990), the biologist Colin Pittendrigh (1918–1996), and the physician and physiologist Jürgen Aschoff (1913–1998). Their pioneering investigations set the stage for contemporary circadian physiology now also known as "chronobiology." Parameters of circadian rhythmicity are mean level, phase, period, amplitude, waveform, and robustness (Fig. 1).

Starting with investigations of Drosophila by Konopka and Benzer (1971), the genetic and molecular basis of circadian rhythm generation and synchronization has been elucidated in animals and plants. These studies led to the identification of "clock genes" that interact in one or more transcriptional/translational feedback loops and whose protein products control the expression of so-called clock-controlled genes.

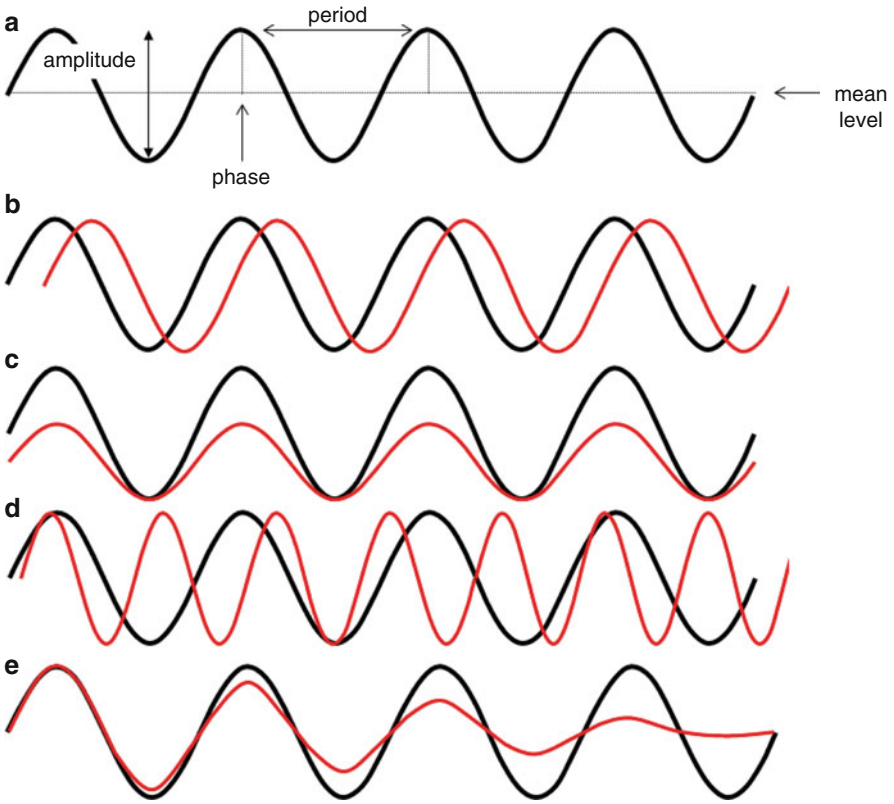

Fig. 1 Characteristics of biological rhythms. Biological rhythms generally follow sine wave forms. (**a**) Rhythmic patterns can be described by the following parameters: period, mean level, amplitude, and phase; (**b**) the phase of the *red curve* is shifted (delayed) in comparison to the *black curve*; (**c**) the amplitude of the *red curve* is lower in comparison to the *black curve*; (**d**) the period length of the *red curve* is shorter, and thus the frequency is higher in comparison to the *black curve*; (**e**) the oscillation of the *red curve* shows weak robustness and damps out after a few cycles

These breakthroughs have established "chronobiology" as a cutting-edge scientific discipline that provides us now with an excellent basis to develop and establish a rational and systematic "chronomedicine" involving a broad range of clinical disciplines from psychiatry to oncology and social medicine.

The Circadian System Is Composed of Circadian Rhythm Generators, Input Pathways, and Output Pathways

Chronobiology has led to the identification and characterization of three core elements that are needed to generate and to maintain rhythmic behavior and functions: (1) endogenous rhythm generator, (2) receptors and input pathways

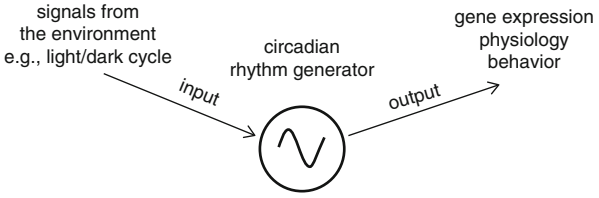

Fig. 2 The three core elements needed to generate and to maintain rhythmic behavior and functions. The circadian rhythm generator generates an endogenous rhythm with a period length of approximately, but not precisely, 24 h. Input pathways transmitting signals from the environment to the circadian rhythm generator (so-called zeitgebers) entrain the endogenous circadian rhythm to the environmental rhythms. Via output pathways, the circadian rhythm generator provides time information required for the control of behavior, physiology, and gene expression

that connect the endogenous rhythm generator(s) with the environment, and (3) output pathways that provide time information required for the control of behavior, physiology, and gene expression (Fig. 2). (1) The endogenous rhythm generators are capable to generate – without any environmental cue – a "free-running" rhythm with a period length of approximately, not precisely, 24 h. Following a proposal of Franz Halberg, another pioneer in the field, such endogenous or free-running rhythms are called "circadian rhythms" (period length: circa the length of one astrophysical day = dies). Circadian rhythms are characterized by two key features: they are self-sustained and they can be entrained to daily changes in the environment. Moreover, circadian rhythm generators are temperature-compensated: at variance with most biochemical reactions which are accelerated at increasing temperature, the period length of the circadian rhythm generators remains rather constant over a wide range of physiological temperatures. (2) Input pathways originate from receptors perceiving and transmitting environmental cues that entrain or synchronize the circadian rhythm generators to the environment. Environmental cues that are able to synchronize circadian rhythm generators, i.e., to shift the phase of the circadian rhythms, are called "zeitgebers." This term has been coined by Jürgen Aschoff, and as one of the few German words, this term made its way directly into the English language. For all living organisms, the change between day and night, the light/dark cycle, is the most important "zeitgeber" by which circadian rhythm generators are entrained to the solar day. The annual cycle of day length change, the photoperiod, represents an important zeitgeber for seasonal rhythms. (3) Output pathways of the circadian rhythm generators transmit signals via multiple effectors. In the case of unicellular organisms, circadian rhythm generators, input pathways, and output pathways necessarily reside in a single cell, but in more complex, multicellular organisms, the three core elements reside in spatially separated cells which need to be interconnected, thus forming a "circadian system."

The Circadian System Has a Hierarchical Architecture and Comprises Central Circadian Rhythm Generators and Subsidiary Clocks in the Periphery

A highly sophisticated circadian system is already present in invertebrates such as arthropods and insects, but the most complex organization of the circadian system is found in mammals and man. Identification of the molecular clockwork has revealed a distinct hierarchy of the circadian system that is composed of central circadian rhythm generators and subsidiary clocks. In invertebrates and vertebrates, the central circadian rhythm generator, also considered as the conductor of the circadian orchestra or the "master clock," resides within the brain. Subsidiary clocks are located in the periphery and can be found in nearly all cells. To maintain rhythmic behavior and physiology at the organismic level, the peripheral clocks need to be coordinated and entrained by the central rhythm generator. The central rhythm generator influences peripheral clocks via multiple pathways. First of all, the central rhythm generator drives rest-activity rhythms that in turn drive feeding rhythms, and the latter provide important synchronizing signals for several peripheral clocks. Moreover, the central rhythm generator influences body temperature rhythms either directly or indirectly through its control of rest-activity cycles, and body temperature rhythms also appear to play an important role for the coordination of peripheral clocks. Finally, the central rhythm generator coordinates peripheral clocks rather directly through neuronal and hormonal output pathways (Fig. 3).

In Mammals the Central Circadian Rhythm Generator Is Located in the Suprachiasmatic Nuclei of the Hypothalamus

In mammals and man, the central circadian rhythm generator is located in the suprachiasmatic nuclei (SCN), bilaterally symmetric assemblies of approximately 10,000 neurons on each side located above the optic chiasm at the basis of the hypothalamus, the basement of the diencephalon (Fig. 4). The daily rhythm of nearly all body functions is disrupted if these nuclei are lesioned by diseases or experiments. Virtually all SCN neurons employ gamma-aminobutyric acid (GABA) as primary transmitter. In addition, SCN neurons contain neuropeptides, such as vasopressin (AVP), vasoactive intestinal polypeptide (VIP), gastrin-releasing peptide (GRP), and calretinin. VIP neurons are concentrated in the ventrolateral part (the "core") of the SCN, and AVP neurons are located in the dorsomedial part (the "shell") of the SCN (for more details, see ▶ Chap. 73, "The Suprachiasmatic Nucleus and the Circadian Timekeeping System of the Body" on the SCN by R. Silver). Maintenance of circadian rhythmicity within the SCN requires communication between SCN neurons. This interneuronal cross talk may involve several transmitters and neuropeptides. Best investigated is the functional significance of

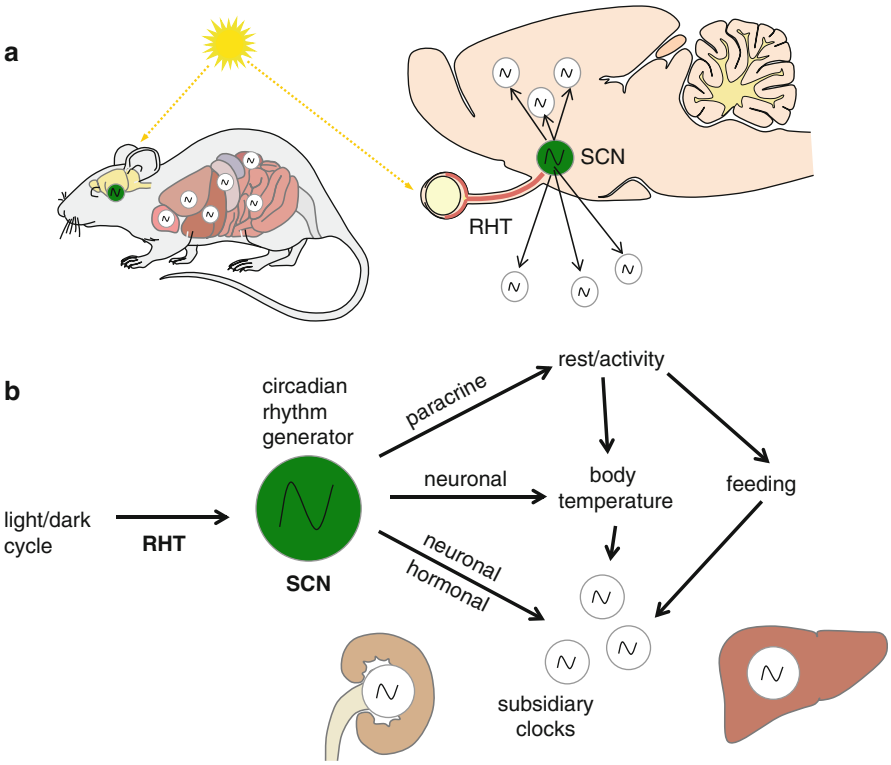

Fig. 3 Hierarchy of the circadian system as shown for mammals. (**a**, **b**) The circadian system is composed of a central circadian rhythm generator and subsidiary clocks. The central circadian rhythm generator, also considered as the conductor of the circadian orchestra or the "master clock," resides within the brain and is entrained by the light/dark cycle. Subsidiary clocks are located in the periphery and can be found in nearly all cells. (**b**) To maintain rhythmic behavior and physiology at the organismic level, the peripheral clocks need to be coordinated and entrained by the central rhythm generator. The central rhythm generator influences peripheral clocks via multiple signaling pathways. Via paracrine signals, the central rhythm generator drives rest-activity rhythms that in turn drive feeding rhythms that provide important synchronizing signals for several peripheral clocks. Moreover, the central rhythm generator influences body temperature rhythms either directly through neuronal signals or indirectly through its control of rest-activity cycles. Like feeding rhythms, body temperature rhythms appear to play an important role for the coordination of peripheral clocks. Finally, the central rhythm generator coordinates peripheral clocks rather directly through neuronal and hormonal output pathways

VIP acting upon the VIP/PACAP 2 receptor. If this receptor is deleted (in transgenic mice), circadian rhythmicity is severely compromised. The molecular basis of circadian rhythm generation is provided by so-called clock genes that interact with each other in transcriptional/translational feedback loops and control the expression of so-called clock-controlled genes.

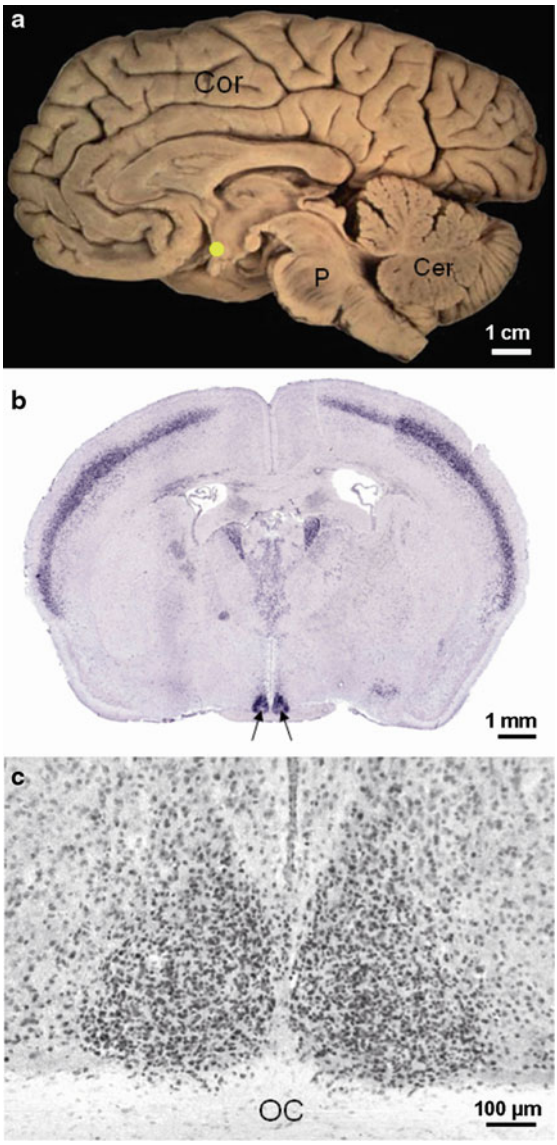

Fig. 4 Location of the central circadian rhythm generator in mammals. The central circadian rhythm generator in mammals is located in the suprachiasmatic nuclei of the hypothalamus (SCN). (**a**) Midsagittal aspect of the human brain. Location of the SCN is indicated by a *yellow* dot, the cortex is labeled by COR, the cerebellum by Cer, and the pons by P. (**b**) Coronal section through the rat brain stained with cresyl violet. *Arrows* point to the paired bilaterally arranged SCN. (**c**) Cytoarchitecture of the rat SCN, the optic chiasm is indicated by OC

Circadian Rhythm Generation in the SCN Involves a Molecular Clockwork

The molecular clockwork is established by clock genes that encode transcription factors containing a basic helix-loop-helix (bHLH) domain involved in DNA binding and a protein dimerization domain called PAS domain (for PER, ARNT, SIM, the founders of the PAS protein family) (Fig. 5). Clock genes and their protein

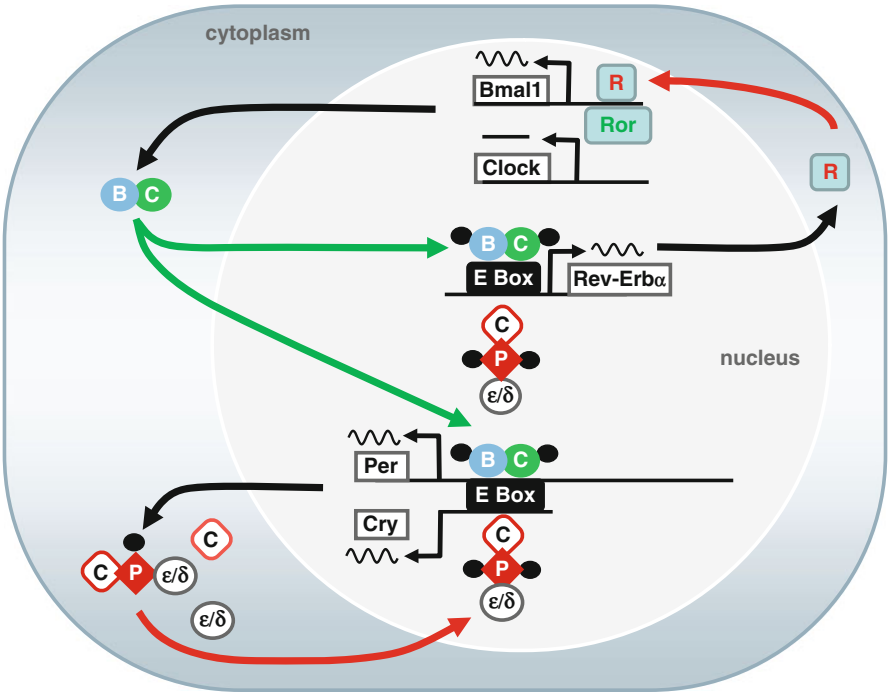

Fig. 5 Principles of the molecular clockwork. *Clock* genes and their protein products interact in autoregulatory transcriptional/translational feedback loops. The core loop comprises the clock genes *Clock* (or *Npas2*, which is not shown here) and *Bmal1* which encode transcription factors containing a basic helix-loop-helix (bHLH) domain involved in DNA binding and a protein dimerization domain called PAS domain. Heterodimers of the basic helix-loop-helix transcription factors CLOCK (C) and BMAL1 (B) form the positive elements, the activator complex. This complex activates the transcription of the *Period* (*Per*) and *cryptochrome* (*Cry*) genes via E-box (like) enhancer elements in their promoters. The protein products of the *Per* (P) and *Cry* (C) form negative regulator (repressor) complexes that also comprise other proteins such as casein kinase 1ε or δ (ε, δ). After translocation into the nucleus, the repressor complex inhibits CLOCK:BMAL1-mediated transcription of the *Per* and *Cry* genes. The repressor complex is removed – at least in part – by ubiquitination and proteasomal degradation, and the negative feedback loop starts again. This primary feedback loop is modulated by accessory feedback loops that involve the orphan nuclear receptors REV-ERBα and RORα that by binding to ROR enhancer elements control the rhythmic activity of the *Bmal1* gene. Importantly, molecular clockworks are not restricted to the central rhythm generator in the SCN but are also present in subsidiary clocks found in a variety of brain regions outside the SCN and in peripheral organs

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repressor complex inhibits CLOCK/NPAS2:BMAL1-mediated transcription of the *Per* and *Cry* genes. The repressor complex is removed – at least in part – by ubiquitination and proteasomal degradation, and the negative feedback loop starts again. This primary feedback loop is modulated by accessory feedback loops that involve the orphan nuclear receptors REV-ERBα and RORα that by binding to ROR enhancer elements (RORE) control the rhythmic activity of the *Bmal1* gene. This molecular clockwork controls (1) the expression of clock-controlled genes (e.g., the gene encoding vasopressin, an important neuropeptide produced in the SCN) and (2) the release of rhythmic SCN output signals. Importantly, molecular clockworks are not restricted to the SCN but are present in a variety of brain regions outside the SCN as well as in peripheral organs ("slave oscillators" or peripheral clocks).

The Central Circadian Rhythm Generator in the SCN Is Entrained by Light

As mentioned above, the daily light–dark cycle, the photoperiod, represents the most important zeitgeber to entrain the circadian rhythm generators to environmental changes. In mammals, information about the photoperiod is exclusively perceived by the retina as has been shown by enucleation studies. Interestingly, photoreception is not confined to the classical retinal photoreceptors, the rods and cones, but is also accomplished by a subset of retinal ganglion cells with the extraordinary capability of responding directly to light. These intrinsically photosensitive retinal ganglion cells (ipRGCs) contain melanopsin as a photopigment, are sensitive to the blue portion of visible light (with a maximum sensitivity at 480 nm), and function as irradiance detectors (Fig. 6). To date, melanopsin-expressing retinal ganglion cells have been demonstrated in several species of mammals including primates and man. Studies with transgenic mice that lack the classical rod and cone photoreceptors have provided convincing evidence that the ipRGCs are essential to mediate entrainment of the circadian rhythm generator in the SCN to the external light–dark cycle. ipRGCs are thus called "circadian" photoreceptors. In nonmammalian vertebrates, i.e., birds, reptiles, amphibians, and fish, "circadian" photoreceptors are also found in "extraretinal" locations, e.g., in the pineal complex of the epithalamus (the roof of the diencephalon) or in the hypothalamus.

The ipRGCs of mammals use glutamate and the neuropeptide PACAP (pituitary adenylyl cyclase activating polypeptide) as transmitters, and their axons form a circumscript portion of the optic nerve called the retinohypothalamic tract (RHT) that terminates in a subdivision of the SCN, the retinorecipient area of the SCN. In addition, ipRGCs project to the intergeniculate leaflet and hypothalamic regions involved in the control of sleep and wakefulness such as the ventrolateral preoptic nucleus (VLPO), the ventral subparaventricular zone (vSPVZ), and the lateral hypothalamic area (LHA). Via projections to the olivary pretectal nucleus (OPN), the ipRCGs also control the pupillary light reflex, thereby regulating the intensity of light entering the eye.

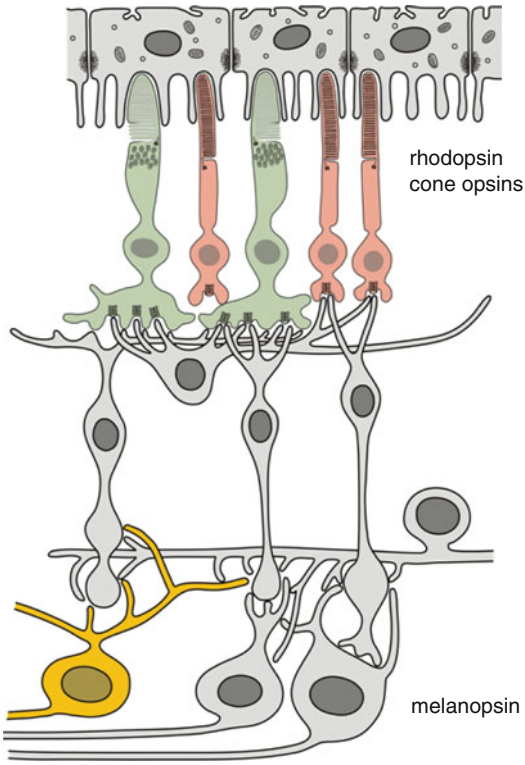

Fig. 6 Schematic diagram of the mammalian retina depicting the different types of photoreceptors. Photoreception is not confined to rods (*red*) and cones (*green*) but is also accomplished by a subset of intrinsically photosensitive retinal ganglion cells (*yellow*), the so-called circadian photoreceptors, that employ melanopsin as a photopigment and give rise to the retinohypothalamic tract. To date, melanopsin-expressing retinal ganglion cells have been demonstrated in several species of mammals including primates and man

The circadian rhythm generator in the SCN is entrained to the environmental day-night changes by making daily adjustments in the phase and period of the circadian oscillator. This can be achieved by short light pulses (lasting a few minutes) given during the dark phase. The generation of phase shifts depends on the time at which they are applied. There is no response to light stimuli applied during day time. Light given in the early night causes phase delays, while light pulses during late night induce phase advances. The fact that the SCN is "sensitive" to light only during certain phases suggests that the SCN controls this input pathway through its molecular clockwork. Glutamate in cooperation with the neuropeptide PACAP appears as the critical neurotransmitter for eliciting phase shifts. At the molecular level, stimulation with light or with glutamate rapidly induces expression of the *Per1* gene via activation of the transcription factor CREB (cyclic AMP response element-binding protein) that activates *Per1* expression by acting upon a cyclic AMP response element (CRE) in the promoter of the *Per1* gene (Fig. 7).

In addition to the RHT, the SCN receives major input from the intergeniculate leaflet (IGL) of the thalamus and the raphe nuclei of the brain stem. The projections

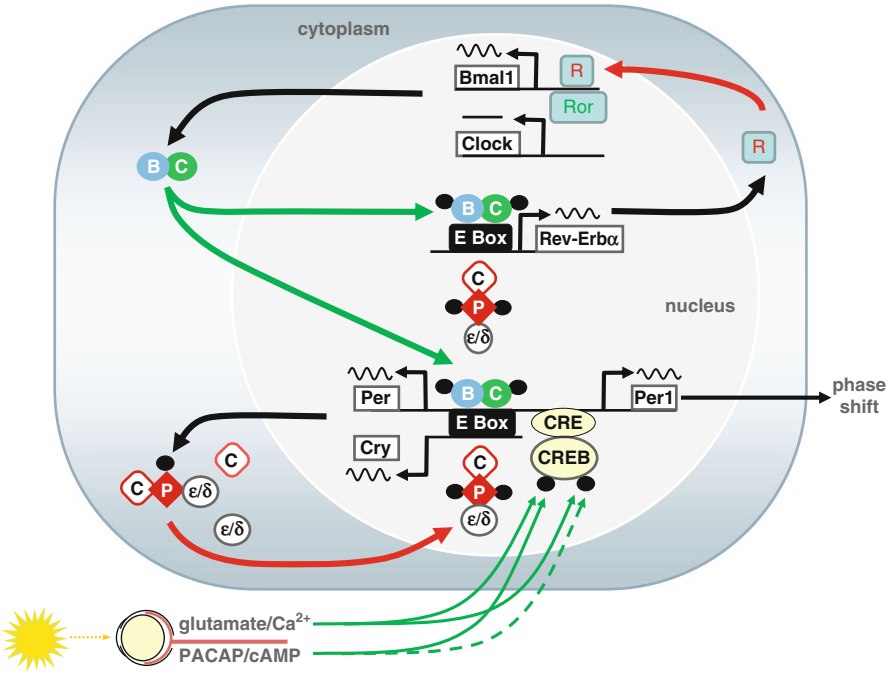

Fig. 7 Molecular mechanisms of entrainment of the central rhythm generator in the SCN. The figure shows the basic principles of the molecular clockwork in the SCN as already depicted in Fig. 5. The most important entrainment signal (zeitgeber) for the SCN is the environmental light/dark cycle which is transmitted to the SCN via the retinohypothalamic tract (RHT). The RHT contains glutamate and PACAP as neurotransmitters. Glutamate in cooperation with the neuropeptide PACAP appears as the critical neurotransmitter for eliciting phase shifts. At the molecular level stimulation with light or with glutamate rapidly induce expression of the *Per1* gene via phosphorylation of the transcription factor CREB (cyclic AMP response element binding protein). Phosphorylated CREB activates *Per1* expression by acting upon a cyclic AMP response element (CRE) in the promoter of the *Per1* gene. Activation of *Per1* expression induces phase shifts. The generation of phase shifts depends on the time at which the light stimuli are applied. There is no response to light stimuli applied during day time. Light given in the early night causes phase delays, while light pulses during late night induce phase advances. The fact that the SCN is "sensitive" to light only during certain phases suggests that the SCN controls this input pathway through its molecular clockwork

from the IGL form the geniculohypothalamic tract (GHT) which employs neuropeptide Y and GABA as neurotransmitters. Since the IGL itself is a target of the RHT (see above), the GHT is supposed to indirectly confer photic information to the SCN which has been processed and possibly delayed in the IGL. The projections from the raphe nuclei confer nonphotic information to the SCN and contain serotonin (5-HT) as neurotransmitter.

Output Pathways of the Central Circadian Rhythm Generator in the SCN Employ Neuronal, Neuroendocrine, and Hormonal Mechanisms

Signals from the SCN are transmitted to other brain regions via (1) neurohormones or paracrine factors which are rhythmically released from SCN neurons into the cerebrospinal fluid and (2) via short-range neuronal projections, most of which remain confined within the boundaries of the hypothalamus where they terminate in various nuclei, e.g., the medial preoptic area, the paraventricular, the subparaventricular, the dorsomedial, and the arcuate nuclei. Apart from the classical neurotransmitters GABA and glutamate, AVP, cardiolipin-like cytokine, prokineticin 2, VIP, and transforming growth factor α have been identified as SCN output signals. Three different types of neurons are identified as targets of the SCN projections: endocrine neurons producing CRH (corticotropin-releasing hormone) and GnRH (gonadotropin-releasing hormone), autonomic neurons sending long descending projections to preganglionic parasympathetic and sympathetic neurons in the brain stem and spinal cord, respectively, and "intermediate" neurons that appear to integrate circadian output with other hypothalamic information and to transmit these integrated signals to endocrine and autonomic neurons (Fig. 8). Notably, the signals emanating from the SCN must be interpreted in an opposite manner in day-active (diurnal) and night-active (nocturnal) animals. Since the organization of the SCN itself appears to be quite similar in nocturnal or diurnal mammals, it may be assumed that the downstream actions of SCN output factors differ between diurnal and nocturnal mammals.

Behavioral, Autonomic, and Endocrine Rhythms Mirror the Functional Activity of the Circadian System

A widely used behavioral readout of the circadian rhythm generator in the SCN is the locomotor activity which can be determined by infrared motion detectors or running wheels (Fig. 9). Apparently, the SCN controls locomotor activity via paracrine or neuroendocrine mechanisms since SCN transplants into the third ventricle of animals whose SCN was previously lesioned restore the locomotor activity rhythms very rapidly, i.e., well before neuronal connections could be reestablished between the transplanted SCN and the hypothalamus of the recipient. In the absence of zeitgebers (e.g., in constant darkness), the locomotor activity follows the circadian rhythm with subjective day and subjective night. In nocturnal rodents, the subjective day is characterized by low and the subjective night by high locomotor activity. Locomotor activity is also a useful parameter to determine phase-shifting effects of zeitgebers and to record phase-response curves. In rodents kept in constant darkness, light stimuli cause a phase delay when applied during the early subjective night and a phase advance when given late during the subjective night. Notably, light stimuli do not affect the phase when given during subjective day. Other rhythmic behavioral functions that are under circadian control include feeding, excretion, sensory

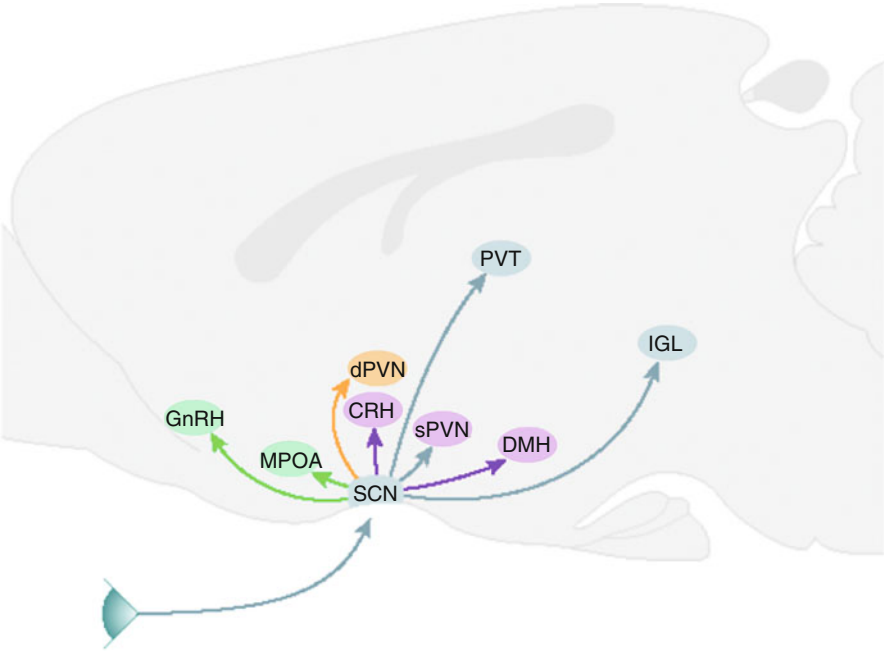

Fig. 8 Neuronal output pathways of the SCN as depicted in a sagittal section through the rodent brain. Neuronal projections terminate in the medial preoptic area (*MPOA*), the dorsal subdivision of the hypothalamic paraventricular nucleus (*dPVN*), the subparaventricular subdivision of the hypothalamic paraventricular nucleus (*sPVN*), the dorsomedial hypothalamic nucleus (*DMH*), the intergeniculate leaflet (*IGL*), and the paraventricular nucleus of the thalamus (*PVT*). Neuronal SCN output pathways also project to endocrine neurons producing corticotropin-releasing hormone (*CRH*) and gonadotropin-releasing hormone (*GnRH*) (After Buijs and Kalsbeek (2001))

processing, and learning capability. The circadian system also influences rhythms of autonomic functions such as the control of body temperature, cardiovascular function, sleep, and the secretion of hormones such as melatonin and glucocorticoids. At variance to the locomotor rhythms, the rhythms in melatonin and glucocorticoids are not restored in SCN-Lesioned animals by SCN transplants. This indicates that the SCN controls the rhythm in these hormones via neuronal output pathways.

Melatonin Is an Important Neuroendocrine Hand of the Circadian System

One of the best characterized output pathway of the SCN is the circuit that connects the hypothalamic SCN with the epithalamic pineal gland and that controls rhythmic melatonin production and secretion (Fig. 10). This circuit comprises GABAergic projections from the SCN to the paraventricular nucleus (PVN) which projects to preganglionic sympathetic neurons in the intermediolateral column of the spinal

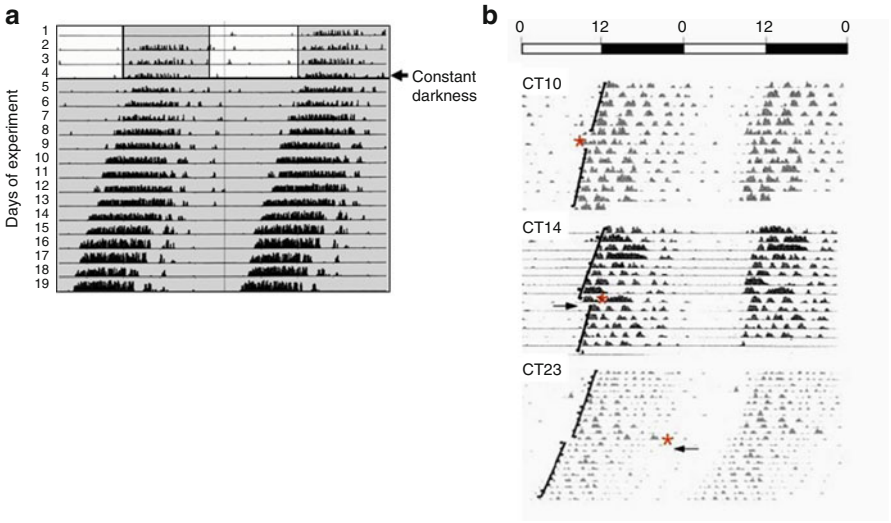

Fig. 9 (**a**, **b**) Double-plotted actograms showing the locomotor activity of mice by black columns. Locomotor activity is recorded by infrared motion detectors or running wheels. (**a**) During the first 4 days of recording, the animals are kept in a 12-h light (*white bar*):12-h dim red light (*gray bar*) cycle. The locomotor activity is entrained to the 24-h light/dim red cycle. On day 5, the animals are transferred to constant dim red light and the endogenous, circadian rhythm generated by the SCN becomes apparent. The period length of the circadian rhythm is shorter than 24 h (it shifts to the left). In nocturnal animals, the phase with high locomotor activity represents the subjective night and the phase with no or very little locomotor activity the subjective day. (**b**) Actograms showing the locomotor activity of mice kept in constant dim red light. The locomotor activity follows the circadian rhythm. At the tenth day in constant dim red light, the animals are subjected to a 15-min light pulse (1,000 lx) at the end of the subjective day (CT = circadian time 10), in the early subjective night (CT 14), and in the late subjective night (CT23). The light pulse given at CT 10 does not affect the phase of the circadian rhythm; the light pulse given at CT 14 resulted in a delayed onset of locomotor activity (i.e., it caused a phase delay); the light pulse given at CT 23 advanced the onset of the locomotor activity (i.e., it caused a phase advance) (Reproduced from Gau et al. (2002))

cord. The latter innervate the sympathetic superior cervical ganglia (SCG). SCG neurons send their axons as postganglionic sympathetic nerve fibers to the pineal gland. These axons contain norepinephrine as primary neurotransmitter which is released from the intrapineal nerve terminals at the beginning of the night, acts upon the pineal specific cells, the pinealocytes, and drives the rhythmic biosynthesis of melatonin by activating the penultimate enzyme of the melatonin biosynthesis, the arylalkylamine N-acetyltransferase (AANAT), via the ß-adrenergic-cyclic AMP pathway. Depending on the species, the activity of AANAT may be controlled via transcriptional and posttranslational processes. An important step for inactivation is the degradation of the AANAT protein by ubiquitination and proteasomal proteolysis (Fig. 11). According to current concepts, the lipophilic neurohormone melatonin is not stored within the pineal gland, but is immediately released into the blood stream and cerebrospinal fluid upon its formation. Since melatonin is only produced

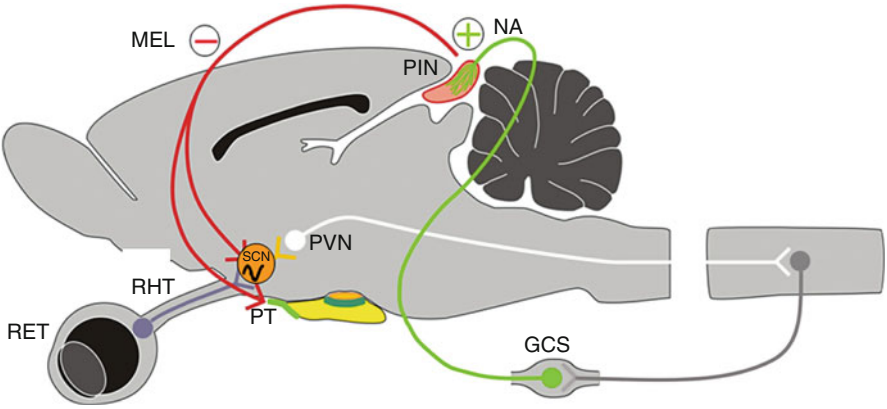

Fig. 10 The circuit connecting the hypothalamic suprachiasmatic nucleus (*SCN*) with epithalamic pineal gland (*PIN*) as depicted in a sagittal plane. This circuit controls rhythmic melatonin production and secretion during night; it comprises GABAergic projections from the SCN (*yellow*) to the paraventricular nucleus (PVN, *white*) which projects to preganglionic sympathetic neurons in the intermediolateral column of the spinal cord (IML, *gray*). The latter innervate the sympathetic superior cervical ganglia (GCS, *light green*). SCG neurons send their axons as postganglionic sympathetic nerve fibers to the pineal gland. These axons contain norepinephrine (NA) as primary neurotransmitter which is released from the intrapineal nerve terminals at the beginning of the night, acts upon the pineal specific cells, the pinealocytes, and drives the rhythmic biosynthesis of melatonin. Via specific membrane-bound receptors, melatonin influences the activity of the SCN and of the pars tuberalis (*PT*). RET indicates retina and RHT retinohypothalamic tract

and secreted during the night, it represents the neuroendocrine message of darkness and an important neuroendocrine hand of the circadian clock. Melatonin production in the pineal gland is also modified by seasonal changes in day lengths; the duration of the melatonin signal increases with the length of the night. Melatonin is thus an important messenger for anticipation and adaptation to seasonal changes of the photoperiod. In addition to the pineal gland, melatonin is also produced in the retina, but retinal melatonin is not secreted into the general circulation, it exerts local effects within the retina.

Light stimuli affect the melatonin biosynthesis in the pineal organ through two distinct mechanisms. Firstly, light at night acutely inhibits melatonin biosynthesis by decreasing AANAT activity. The intensity of the light required to suppress melatonin biosynthesis varies from species to species, but generally, blue light is more effective than red light. Such acute effects of light that inhibit expressed rhythms, but do not cause entrainment of the circadian oscillator, are called “masking.” Secondly, light stimuli affect the circadian rhythm generated in the SCN in a phase-dependent manner; light stimuli applied in the first part of the night delay the rhythm, and light stimuli given in the second part of the night advance the rhythm.

Melatonin affects several target tissues which are equipped with high-affinity melatonin receptors. Two subtypes of melatonin receptors have been identified in mammals and are denominated as MT1 (Mel1a) and MT2 (Mel1b) receptors. A third type of melatonin receptor denominated as Mel1c is found only in nonmammalian

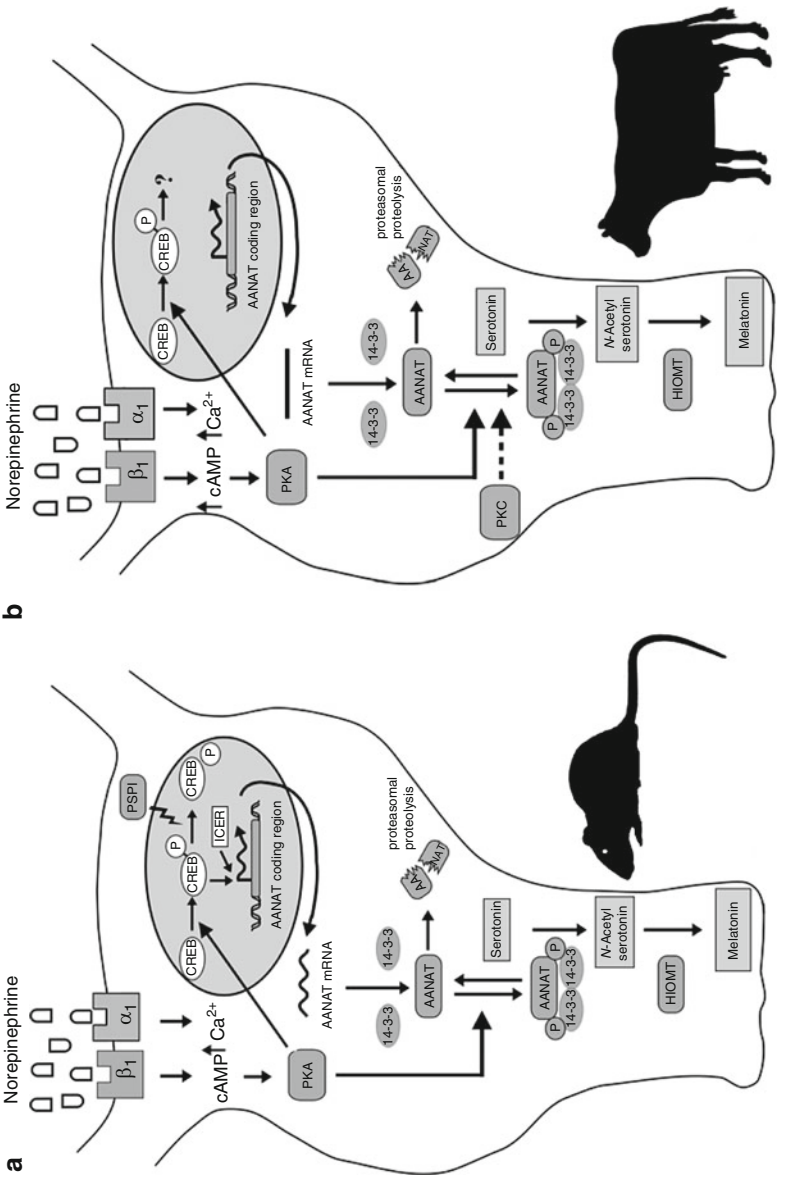

Fig. 11 (continued)

vertebrates. Melatonin receptors belong to the superfamily of G-protein-coupled receptors. The MT1 receptor is coupled to different G proteins that mediate inhibition of adenylyl cyclase and activation of phospholipase Cβ. The MT2 receptors mediate inhibition of adenylyl cyclase and of soluble guanylyl cyclase. Various agonists and antagonists have been developed to study the functional significance of the melatonin receptors. Notably, three high-affinity agonists, agomelatine, ramelteon, and tasimelteon, appear to be of clinical relevance in humans and are used for the treatment of major depression and insomnia, diseases that are considered to be associated with malfunction of the circadian system.

As compared to nonmammalian vertebrates, melatonin receptors are less widely distributed in mammals. In these species, the highest density of melatonin receptors is found in the pars tuberalis of the hypophysis (Fig. 12). Furthermore, MT1 receptors are localized in the hypothalamus, particularly in the SCN, the cerebral cortex, cerebellum, thalamus, and hippocampus. Melatonin receptors are also expressed in peripheral tissues, such as the adrenal gland, arteries, heart, liver, lung, small intestine, skin, and T and B lymphocytes.

Fig. 11 Important steps of the melatonin biosynthesis in mammalian pinealocytes. Norepinephrine (NE) released from sympathetic nerve fibers during night is the primary neurotransmitter that controls melatonin biosynthesis in mammals. NE acts upon two subtypes of adrenergic receptors. Of utmost importance is the activation of β_1-adrenergic receptors which leads to increases in intracellular cyclic AMP concentration and causes activation of cyclic AMP-dependent protein kinase A (*PKA*). This cascade stimulates the penultimate enzyme of the melatonin biosynthesis, the arylalkylamine N-acetyltransferase (*AANAT*), and is indispensible for the activation of melatonin biosynthesis. NE also acts upon α-adrenergic receptors and their activation causes increases in the intracellular calcium ion concentration. The functional significance of this cascade is less clear and it may vary with the species investigated. The downstream mechanisms which link the β_1-adrenergic-cyclic AMP-PKA pathway to activation of AANAT show striking variation between rodents (**a**) and ungulates and primates (**b**). In rodents (**a**), the pathway controls transcriptional mechanisms to regulate melatonin synthesis: it leads to phosphorylation of the transcription factor cyclic AMP response element-binding protein (*CREB*). Via binding to a cyclic AMP response element (CRE) in the promoter of the *Aanat* gene phosphorylated CREB enhances *Aanat* transcription resulting in a dramatic rise in *Aanat* mRNA levels followed by increases in AANAT protein levels and enzyme activity. Transcription of the *Aanat* gene is reduced by dephosphorylation of CREB through the protein serine/threonine phosphatase 1 (*PSP1*) and increased concentrations of the inhibitory transcription factor inducible cyclic AMP early repressor (*ICER*) that interacts with phosphorylated CREB. At variance to rats and mice, *Aanat* mRNA levels are not rhythmic in ungulates and primates (**b**). In rodents (**a**) as well as in ungulates and primates (**b**), the cyclic AMP-PKA pathway also controls degradation of the AANAT protein by proteasomal proteolysis. When cyclic AMP levels are low, newly formed AANAT protein is immediately degraded by the proteasome. Increases in cyclic AMP levels followed by activation of PKA cause phosphorylation of the AANAT protein. Phosphorylated AANAT protein interacts with 14-3-3 proteins and thus becomes protected from proteasomal proteolysis and activated. Hydroxyindole-O-methyltransferase (*HIOMT*) is the final enzyme of the melatonin biosynthesis. According to current concepts, the lipophilic neurohormone melatonin is not stored within the pineal gland, but is immediately released into the blood stream and cerebrospinal fluid upon its formation. Since melatonin is only produced and secreted during the night, it represents the neuroendocrine message of darkness and an important neuroendocrine hand of the circadian clock (Reproduced from Schomerus and Korf (2005))

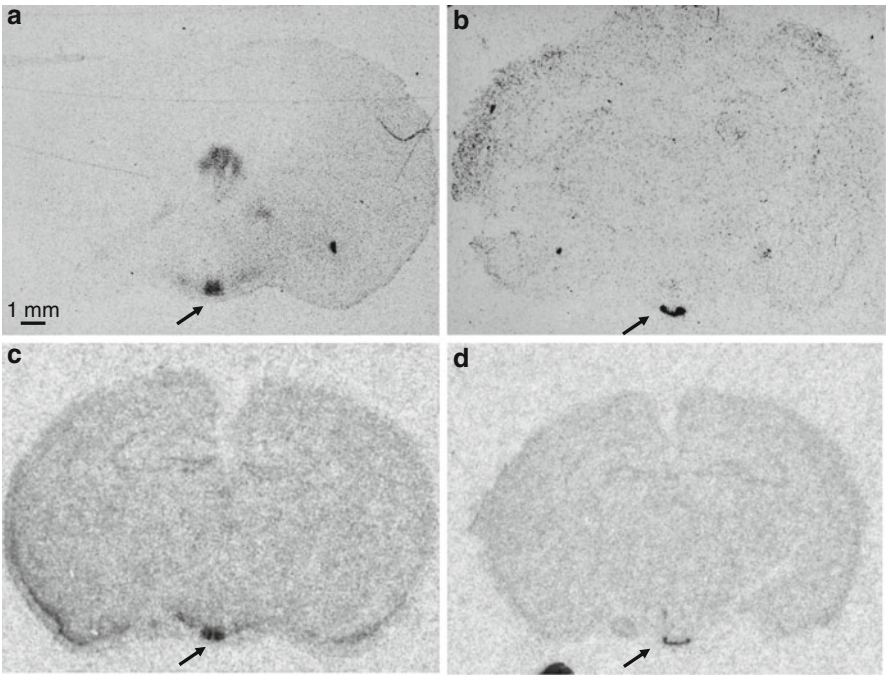

Fig. 12 Melatonin targets in the rodent brain. Coronal sections through the mouse brain show iodomelatonin binding sites in the SCN (**a**, *arrow*) and hypophysial pars tuberalis (**b**, *arrow*) and in situ hybridization signals for the melatonin receptor 1 (MT1) mRNA in the SCN (**c**, *arrow*) and hypophysial pars tuberalis (**d**, *arrow*)

Melatonin Affects the Activity of the SCN

Melatonin provides an important time cue during the prenatal and early postnatal development when the retinohypothalamic tract and the neural connections between the SCN and the pineal organ are not yet fully matured. Melatonin derived from the mother readily passes the placental barrier, and after birth, the hormone is taken up with the milk to serve as communicator of the ambient light/dark conditions until the pup's retinohypothalamic tract and pineal organ have gained full function. The effects of melatonin on the adult circadian system are more subtle; they appear to be associated with sleep propensity and the core body temperature rhythm. Direct effects of melatonin on the SCN have been shown by in vitro experiments. Via activation of the MT1 receptor, melatonin acutely inhibits neuronal firing of SCN neurons. Moreover, depending on the time point of application, melatonin is capable of phase-shifting the rhythm of neuronal firing rate of the SCN and the locomotor behavior (Fig. 13) of the animals. Because of these phase-shifting effects, melatonin has been considered as "chronobiotic" involved in the entrainment of the SCN together with other signals originating, e.g., from the retinohypothalamic tract. The phase-shifting effects of melatonin are very obvious in blind humans whose

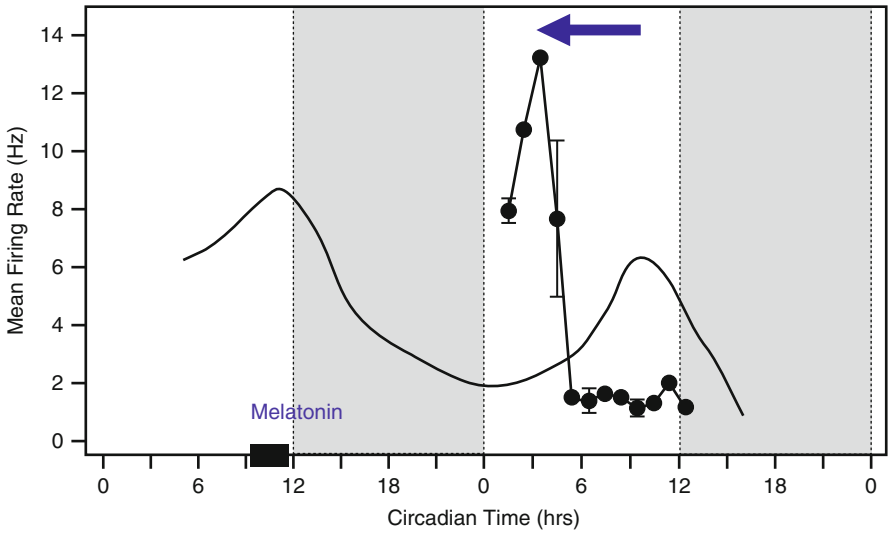

Fig. 13 Effects of melatonin in the rat SCN. Melatonin applied to SCN slice cultures at the end of the subjective day phase advances the rhythm in neuronal firing rate (Figure courtesy of H. Meissl, Frankfurt. Reproduced from Korf and von Gall (2006))

retinohypothalamic tract is destroyed. Interestingly, studies with mice provided evidence that melatonin acting upon the SCN elicits inhibitory effects on the sympathetic nervous system and may thus have a stress-reducing potential.

Melatonin Is a Seasonal Time Cue and Controls the Hypophysial Pars Tuberalis

Melatonin secretion is related to the length of the night: the longer the night, the longer is the duration of melatonin secretion. Melatonin is thus an important seasonal time cue for the control of body functions that vary with the season, such as reproduction, pelage (coat growth and color), appetite, body weight, and sleep. These seasonal effects are brought about by the action of melatonin on the hypophysial pars tuberalis. The pars tuberalis represents an important regulatory center for neuroendocrine and endocrine functions within the hypothalamohypophysial system which is a master regulator of endocrine coordination. The hypothalamus integrates environmental and metabolic signals and contains a variety of neuroendocrine neurons that produce neurohormones and release them from their axon terminals in the median eminence into the portal vasculature of the hypophysis. These hypothalamic neurohormones control the function of endocrine cells located in the pars distalis of the hypophysis and function as either releasing or inhibiting factors. Releasing factors stimulate and inhibiting factors suppress hormone

secretion from endocrine hypophysial cells. Hypophysial hormones such as TSH, ACTH, FSH, LH, prolactin, and somatostatin influence endocrine organs and other peripheral tissues. The pars tuberalis is the rostral part of the anterior pituitary and comprises specific cells that can be distinguished from the endocrine cells in the pars distalis. The PT is in close vicinity to the external layer of the median eminence, the portal vasculature, and the hypophysial pars distalis. The PT is connected with the third ventricle partially via processes of tanycytes in the infundibular recess of the third ventricle and the median eminence. The PT-specific cells contain high levels of MT1 receptors that are essential to transmit the melatonin signal to the PT. The signal transduction cascades between melatonin receptors and output pathways of the pars tuberalis involve a PT-intrinsic molecular clockwork whose rhythm is not self-sustained but driven by the melatonin signal.

Notably, the pars tuberalis sends its signals in two directions: via a "retrograde" pathway to the hypothalamus and via an "anterograde" pathway to the pars distalis (Fig. 14). Thyroid- stimulating hormone (TSH) has been identified as a key messenger of the retrograde pathway. TSH is induced in the pars tuberalis when the nights and thus the melatonin signal become shorter (i.e., under long-day condition). TSH acts upon ependymal cells (tanycytes) that line the infundibular recess of the third ventricle and express the TSH receptor as well as deiodinase type 2 and 3 (DIO2 and DIO3). DIO2, the enzyme that converts the prohormone thyroxine (T4) to bioactive T3, is upregulated under long-day conditions, and this upregulation leads to a local increase of T3 in the mediobasal hypothalamus. Conversely, DIO3 that inactivates T3 is suppressed under long-day condition. These reciprocal responses of DIO2 and DIO3 efficiently accelerate the local accumulation of T3 that appears to activate the gonadal axis by affecting neuroglial interactions between GnRH nerve terminals and tanycytes in the median eminence. This hypothalamic thyroid hormone signaling system is also found in birds; it thus appears conserved to govern seasonal biology in vertebrates. However, the mechanisms that control this system fundamentally differ between birds and mammals: in birds, the functional activity of the system is not controlled by the melatonin signal, but via photoreceptors that are located in the hypothalamus.

Via the "anterograde" pathway, the pars tuberalis directly controls the function of the pars distalis. Best investigated is the impact of the pars tuberalis on prolactin secretion which increases under long-day conditions. The substances that mediate the anterograde signals from the pars tuberalis to the pars distalis have not yet been fully elucidated. The search was for a long time focused on neuropeptides, and several candidates have been suggested including tachykinins. Recent studies with hamsters have shown that the PT comprises an intrinsic endocannabinoid system which is regulated by the photoperiod. These results indicate that the PT also synthesizes lipidergic messengers such as the endocannabinoids. To date, 2-arachidonoylglycerol (2-AG) appears as the most important lipidergic messenger from the PT which is upregulated under long-day conditions. The primary target of 2-AG is the cannabinoid receptor type 1 (CB1). This receptor is expressed by various cell types in the pars distalis, suggesting that PT-derived endocannabinoids affect a variety of endocrine cells in the hypophysis.

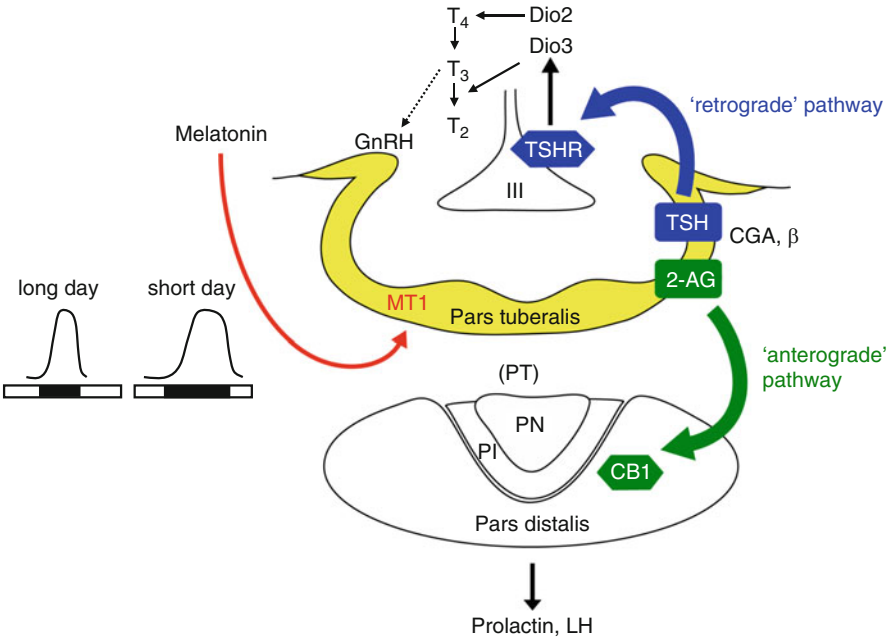

Fig. 14 Signaling pathways and location of the rodent hypophysial pars tuberalis (*PT*) in coronal plane. Photoperiodic information is transformed into melatonin signals (long days and short nights result in a short melatonin signal; short days and long nights result in a long melatonin signal). Melatonin acts upon melatonin receptor 1 (*MT1*) expressed in the PT in high density. The PT sends signals in two directions: via the "retrograde" pathway to the hypothalamus and via the "anterograde" pathway to the hypophysial pars distalis (*PD*). Thyrotropin (*TSH*), composed of common glycoprotein subunit α (*CGA*) and the β-subunit of thyrotropin (β), is a messenger in the retrograde pathway which is upregulated under long-day conditions. It acts upon TSH receptors (*TSHR*) located in the ependymal layer of the third ventricle (III) and controls the expression of type 2 and type 3 deiodinase (Dio2, Dio3) that regulate the local concentration of thyroid hormones (T4, T3, T2). Increased expression of *Dio2* causes increases in T3 levels that stimulate gonadotropin-releasing hormone (*GnRH*) secretion and thus cause an increase in luteinizing hormone (*LH*) secretion from the hypophysial pars distalis (*PD*). The endocannabinoid 2-arachidonoylglycerol (2-AG) is a messenger in the anterograde pathway which is upregulated under long-day conditions. The primary target of 2-AG is the cannabinoid receptor type 1 (*CB1*). This receptor is expressed by various cell types in the pars distalis, suggesting that PT-derived endocannabinoids affect a variety of endocrine cells in the hypophysis including the prolactin-producing cells. Pars intermedia (*PI*), pars nervosa (*PN*) (Reproduced from Yasuo and Korf (2011))

Melatonin Affects Various Physiological Processes

In humans, melatonin has profound effects on temperature regulation and alertness; these effects are discussed under the sections "The Circadian System Influences the Sleep-Wake Cycle" and "The Circadian System Is Involved in the Control of Body Temperature." Via activation of the MT1 receptor, melatonin causes vasoconstriction of the rat caudal artery, while activation of the MT2

receptor resulted in vasodilatation. This effect is suggested to be responsible for the increase in blood flow in the distal parts of the skin and heat loss regulation and may thus underlie the hypothermic effects of the hormone (see below). Melatonin may influence insulin production in the endocrine pancreatic islets and modulate pain sensation.

Glucocorticoids Provide Circadian Information

In addition to melatonin, glucocorticoids show a strong daily oscillation in both laboratory rodents and humans and represent an important phase entrainment signal within the circadian system. Glucocorticoids are hormones secreted from the cortex (zona fasciculata) of the adrenal gland and act on glucocorticoid receptors which are widely distributed in the body, except for the SCN where they are absent. Glucocorticoids influence glucose and protein metabolism and have anti-inflammatory properties. In humans, cortisol is the most important glucocorticoid, whereas the adrenal cortex of many rodents almost exclusively secretes corticosterone. The secretion of adrenal glucocorticoids is regulated by the hypothalamo-pituitary-adrenal gland (HPA) axis (Fig. 15). The HPA axis controls the reaction to stress and regulates many functional systems including the digestive and the immune system. Glucocorticoid synthesis and release from the adrenal cortex is stimulated by ACTH (adrenocorticotropic hormone) secreted from the corticotrope cells of the anterior lobe (pars distalis) of the hypophysis. ACTH levels are controlled by corticotropin-releasing hormone (CRH) and increase prior to waking. CRH is produced by endocrine neurons in the hypothalamus (the medial parvicellular neurons in the PVH) and released into the portal vasculature of the hypophysis. Glucocorticoids feed back to the corticotropes and CRH-producing neurons in the hypothalamus. The CRH neurons receive information from the central circadian rhythm generator in the SCN by means of neuronal projections (see above). Vasopressinergic SCN output signals inhibit CRH release from the PVN and thus control the diurnal glucocorticoid (GC) rhythm. In mice with a corrupted molecular clockwork, the regulation of the hypothalamo-pituitary-adrenal (HPA) axis is defective. Moreover, the molecular clockwork in the adrenal gland, a subsidiary clock, gates glucocorticoid production in response to adrenocorticotropin and controls rhythmic expression of a variety of genes involved in corticosterone biosynthesis. Glucocorticoid levels reach peak values during wake time; in humans the serum concentration starts to rise in the second part of the night, reaches maximal values between 06.00 and 08.00 a.m., and falls during the day.

Glucocorticoids have been shown to synchronize various peripheral tissues, such as liver, kidney, and heart, while other peripheral clocks do not respond. This difference underlines the concept that the circadian system employs multiple timing signals and that each peripheral clock requires a specific combination of synchronizing signals.

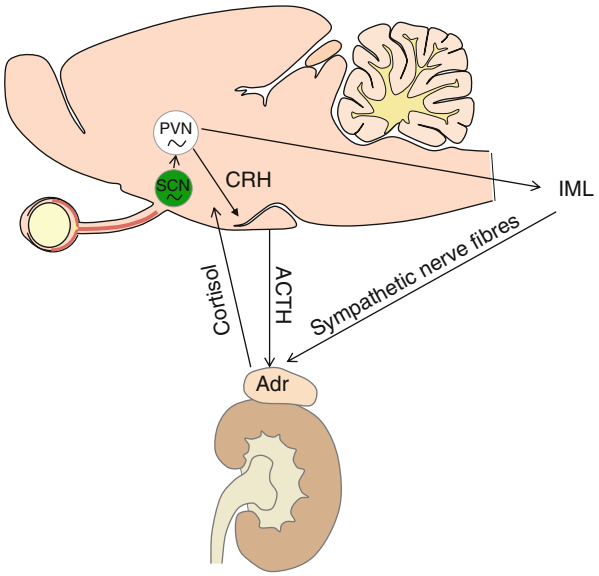

Fig. 15 Regulation of glucocorticoid levels. Glucocorticoids show a strong daily oscillation in both laboratory rodents and humans and represent an important entrainment signal within the circadian system. Glucocorticoids are hormones secreted from the cortex (zona fasciculata) of the adrenal gland (*Adr*) and act on glucocorticoid receptors which are widely distributed in the body, except for the SCN where they are absent. In humans, cortisol is the most important glucocorticoid, whereas the adrenal cortex of many rodents almost exclusively secretes corticosterone. The secretion of adrenal glucocorticoids is regulated by the hypothalamo-pituitary-adrenal gland (HPA) axis. Glucocorticoid synthesis and release from the adrenal cortex is stimulated by ACTH (adrenocorticotropic hormone) secreted from the corticotrope cells of the anterior lobe (pars distalis) of the hypophysis. ACTH levels are controlled by corticotropin-releasing hormone (*CRH*) and increase prior to waking. CRH is produced by endocrine neurons in the hypothalamus (the medial parvicellular neurons in the PVN) and released into the portal vasculature of the hypophysis. Glucocorticoids feed back to the corticotropes and CRH-producing neurons in the hypothalamus. The CRH neurons receive information from the central circadian rhythm generator in the SCN by means of neuronal projections. In addition, the functional activity of the adrenal gland is influenced by sympathetic nerve fibers originating from the intermediolateral column (*IML*) of the spinal cord that receives projections from the paraventricular nucleus (*PVN*)

The Circadian System Influences the Sleep-Wake Cycle

The timing of sleep is controlled by the circadian system which is interconnected with homeostatic sleep propensity (the need for sleep as a function of the amount of time elapsed since the last adequate sleep episode). In a current model, the SCN alternates between actively promoting wake and sleep at opposite phases of its daily cycle. Sleep-promoting cells are concentrated in the preoptic anterior hypothalamus, more specifically in the VLPO, the median preoptic nucleus (MnPO), and the MPO. VLPO neurons are most active during sleep and promote sleep via inhibitory

GABAergic and galaninergic projections to arousal-related monoaminergic cell groups such as the histaminergic tuberomammillary nucleus (TMN), the noradrenergic locus coeruleus (LC), and the serotonergic dorsal raphe nuclei. Conversely, noradrenaline and serotonin inhibit GABAergic neurons in the VLPO, creating a positive feedback loop which stabilizes sleep/wake states.

The sleep-promoting cell groups in the preoptic anterior hypothalamus are reciprocally connected but receive only few direct SCN projections. However, the VLPO has a strong indirect SCN projection via the SPVZ. Especially the ventral SPVZ plays a key role in the control of the sleep/activity rhythm. In addition, the VLPO receives heavy input from the DMH which receives direct and indirect SCN input.

Wake-promoting cells are predominantly concentrated in the lateral hypothalamic area (LHA). Orexin (hypocretin)-containing cells in the LHA are active in association with wake and/or locomotor activity and play an important role in consolidation of sleep-wake states and in the control of energy metabolism. Orexin-containing neurons project to arousal-related monoaminergic nuclei, including the dorsal raphe nuclei (serotonin), the LC (norepinephrine), and the ventral tegmental area (dopamine). These projections may account for the altered sensitivity to psychostimulants in mice lacking orexin. Orexin activates the neurons of arousal-related areas such as the LC and suppresses activity in the VLPO. In addition, orexin-containing nerve fibers project to the SCN and orexin is able to modulate the activity pattern of SCN neurons. Thus, orexin might act as a reset mechanism for sleep/wake cycles and can possibly convey arousal information to the SCN. The LHA receives few direct SCN projections, but the DMH which receives direct and indirect (via SPVZ) SCN projections strongly innervates the LHA. In addition, the endogenous sleep factor adenosine is implicated in promoting sleep via A_1 receptors in the LHA.

The sleep-promoting and wake-promoting hypothalamic brain regions are closely interconnected with each other. The SPVZ and the DMH seem to act as important integrators for circadian timing information from the SCN with other nonrhythmic inputs. Lesion of either the ventral SPVZ or the DMH leads to a loss of circadian rest-activity rhythms. The sleep- and wake-promoting hypothalamic brain regions receive inhibitory and excitatory DMH projections, respectively. DMH projections to the VLPO are predominantly GABAergic, whereas DMH projections to the LHA contain glutamate and TRH. However, lesions of the DMH lead to a marked reduction in total daily wake time, suggesting an overall physiological influence of the DMH.

Although there is evidence that both stimulatory and inhibitory signals from the SCN actively promote both wake and sleep at different phases of the 24-h L/D cycle, no SCN factors promoting arousal have been identified yet. In nocturnal rodents, the SCN releases diffusible factors, including transforming growth factor-a, prokineticin-2, and cardiotrophin-like cytokine which inhibit activity during the daily sleep period (Fig. 16).

In humans, the nocturnal melatonin peak is closely associated with maximum tiredness/fatigue and lowest alertness and performance. Exogenous application of melatonin during the day time acutely increases sleepiness, while suppression of the melatonin peak by light during night was paralleled by increases in alertness and

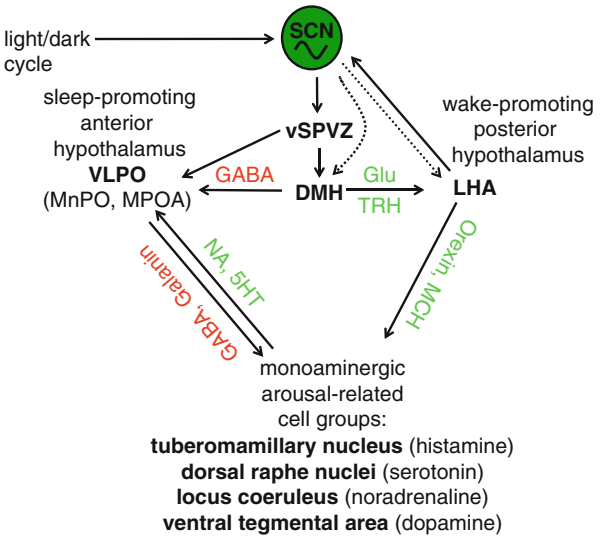

Fig. 16 Major brain centers controlling the sleep-wake cycle. The timing of sleep is controlled by the circadian system which is interconnected with homeostatic sleep propensity (the need for sleep as a function of the amount of time elapsed since the last adequate sleep episode). In a current model, the SCN alternates between actively promoting wake and sleep at opposite phases of its daily cycle. Sleep-promoting cells are concentrated in the preoptic anterior hypothalamus: in the ventrolateral preoptic area (*VLPO*), the median preoptic nucleus (*MnPO*), and the medial preoptic area (*MPOA*). VLPO neurons are most active during sleep and promote sleep via inhibitory GABAergic and galaninergic projections to arousal-related monoaminergic cell groups such as the histaminergic tuberomammillary nucleus (*TMN*), the noradrenergic locus coeruleus (*LC*), and the serotonergic dorsal raphe nuclei. Conversely, noradrenaline and serotonin inhibit GABAergic neurons in the VLPO, creating a positive feedback loop which stabilizes sleep/wake states. The sleep-promoting cell groups in the preoptic anterior hypothalamus are reciprocally connected but receive only few direct SCN projections. However, the VLPO has a strong indirect SCN projection via the subparaventricular zone (*SPVZ*). Especially the ventral subparaventricular zone (*vSPVZ*) plays a key role in the control of the sleep/activity rhythm. In addition, the VLPO receives heavy input from the dorsomedial nucleus of the hypothalamus (*DMH*) which receives direct and indirect SCN input. Wake-promoting cells are predominantly concentrated in the lateral hypothalamic area (*LHA*). Orexin (hypocretin)-containing cells in the LHA are active in association with wake and/or locomotor activity and play an important role in consolidation of sleep-wake states and in the control of energy metabolism. Orexin-containing neurons project to arousal-related monoaminergic nuclei, including the dorsal raphe nuclei (serotonin), the locus coeruleus (norepinephrine), and the ventral tegmental area (dopamine). These projections may account for the altered sensitivity to psychostimulants in mice lacking orexin. Orexin activates the neurons of arousal-related areas such as the locus coeruleus and suppress activity in the VLPO. In addition, orexin-containing nerve fibers project to the SCN and orexin is able to modulate the activity pattern of SCN neurons. Thus, orexin might act as a reset mechanism for sleep/wake cycles and can possibly convey arousal information to the SCN. The LHA receives few direct SCN projections, but the DMH which receives direct and indirect (via SPVZ) SCN projections strongly innervates the LHA. The sleep-promoting and wake-promoting hypothalamic brain regions are closely interconnected with each other. The SPVZ and the DMH seem to act as important integrators for circadian timing information from the SCN with other nonrhythmic inputs. Lesion of either the vSPVZ or the DMH leads to a loss of circadian rest-activity rhythms. The sleep- and wake-promoting hypothalamic brain regions receive inhibitory and excitatory DMH projections, respectively. DMH projections to the VLPO are predominantly GABAergic, whereas DMH projections to the LHA contain glutamate and TRH

performance and a decrease in sleepiness. Most likely, the sleepiness-inducing potential of melatonin may depend on the changes in the core body temperature.

The Circadian System Is Involved in the Control of Body Temperature

There are two hypotheses with regard to the impact of the circadian system on daily changes of the body temperature. The first hypothesis considers that changes of body temperature are primarily under homeostatic control, but are modulated by the circadian system through daily oscillations in the thermoregulatory set point. The other hypothesis assumes that the changes are primarily under circadian control and secondarily modulated by the thermoregulatory system.

Complex populations of cells that control body temperature, sleep, and reproduction are located in the preoptic area. Lesions in the ventromedial preoptic nucleus, avoiding the VLPO and medial-extended VLPO, caused changes in circadian rhythms in body temperature without affecting sleep. This suggests that although neurons involved in sleep and thermal regulation share considerable input and interact extensively, the sites that ultimately regulate these physiological responses are anatomically and functionally separable. Lesions of the dorsal SPVZ lead to a loss in circadian rhythms in body temperature but have virtually no effect on activity rhythms. The SPVZ projects to the preoptic area and the DMH. Lesions of the DMH lead to a reduction of body temperature and also to a reduction in circadian rhythm amplitude. This is consistent with the prominent role of the SPVZ and the DMH as important integrators for circadian timing information (Fig. 17).

In humans, melatonin, an important neuroendocrine hand of the circadian system, appears to be a major regulator of core body temperature. The nocturnal melatonin peak is closely associated with the nadir in body temperature, and exogenous application of melatonin during the day time acutely decreases core body temperature, while suppression of the melatonin peak by light during night was paralleled by increases in body temperature. Most likely, the hypothermic effects of melatonin depend on melatonin-induced vasodilatation and an increased blood flow in the skin of the distal extremities.

The Circadian System Is Involved in the Control of the Female Reproductive Cycle

Intact SCN are essential for maintenance of the reproductive cycle in females. Lesions of the SCN lead to a disruption of estrous cyclicity and prevent the preovulatory estradiol (E2)-induced surge of gonadotropin-releasing hormone (GnRH) from the hypothalamus. The GnRH surge is a prerequisite for the surge of luteinizing hormone (LH) from the anterior pituitary and initiates the ovulation via the hypothalamo-pituitary-ovarian axis. Vasopressin- and VIPergic SCN output projects directly to GnRH- and estrogen receptor (ER)-containing neurons in the preoptic area. In particular, the anteroventral periventricular nucleus (AVPV) of the

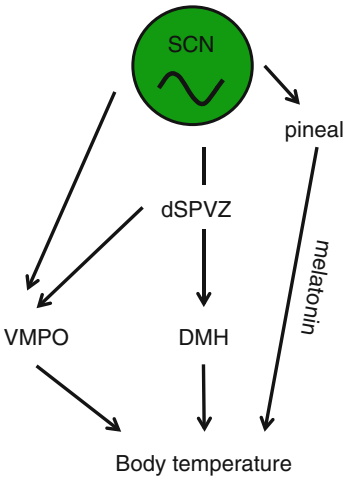

Fig. 17 Major brain areas controlling body temperature. There are two hypotheses with regard to the impact of the circadian system on daily changes of the body temperature. The first hypothesis considers that changes of body temperature are primarily under homeostatic control, but are modulated by the circadian system through daily oscillations in the thermoregulatory set point. The other hypothesis assumes that the changes are primarily under circadian control and secondarily modulated by the thermoregulatory system. Complex populations of cells that control body temperature, sleep, and reproduction are located in the preoptic area. Lesions in the ventromedial preoptic nucleus (*VMPO*), avoiding the ventrolateral preoptic area, caused changes in circadian rhythms in body temperature without affecting sleep. This suggests that although neurons involved in sleep and thermal regulation share considerable input and interact extensively, the sites that ultimately regulate these physiological responses are anatomically and functionally separable. Lesions of the dorsal subparaventricular zone (*dSPVZ*) lead to a loss in circadian rhythms in body temperature but have virtually no effect on activity rhythms. The SPVZ projects to the preoptic area and the dorsomedial nucleus of the hypothalamus (*DMH*). Lesions of the DMH lead to a reduction of body temperature and also to a reduction in circadian rhythm amplitude. This is consistent with the prominent role of the SPVZ and the DMH as important integrators for circadian timing information. In humans, melatonin appears to be a major regulator of core body temperature. The nocturnal melatonin peak is closely associated with the nadir in body temperature, and exogenous application of melatonin during the day time acutely decreases core body temperature, while suppression of the melatonin peak by light during night was paralleled by increases in body temperature

preoptic region is critical for the GnRH/LH surge. This region expresses a high density of ER, projects to GnRH neurons, and receives neural input from the SCN (Fig. 18).

The Circadian System Is Involved in the Control of Food Intake

Circadian and homeostatic regulation of food intake and metabolism are controlled by various hypothalamic brain regions interacting with the brain stem and higher cortical centers (Fig. 19). The arcuate nucleus (Arc) plays a central role in the

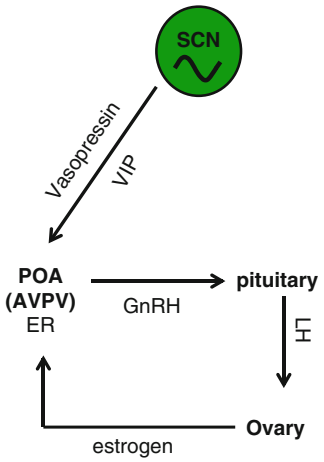

Fig. 18 Interaction between the suprachiasmatic nuclei (*SCN*) and the reproductive system in females. Lesions of the SCN lead to a disruption of estrous cyclicity and prevent the preovulatory estradiol (E2)-induced surge of gonadotropin-releasing hormone (*GnRH*) from the hypothalamus. The GnRH surge is a prerequisite for the surge of luteinizing hormone (*LH*) from the anterior pituitary and initiates the ovulation via the hypothalamo-pituitary-ovarian axis. Vasopressin- and VIPergic SCN output projects directly to GnRH- and estrogen receptor (*ER*)-containing neurons in the preoptic area (*POA*). In particular, the anteroventral periventricular nucleus (*AVPV*) of the preoptic region is critical for the GnRH/LH surge. This region expresses a high density of ER, projects to GnRH neurons and receives neural input from the SCN

regulation of hunger and satiety. The Arc has the ability to monitor the peripheral metabolic status through hormones such as ghrelin, insulin, and leptin and through metabolites such as fatty acids and glucose via the blood stream. The Arc projects to the PVN, the DMH, the LHA, and the VMH. Neurons in the Arc which coexpress neuropeptide Y (NPY) and agouti-related peptide (AgRP) stimulate food intake (they are orexigenic), whereas neurons coexpressing pro-opiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART) suppress feeding (they are anorexigenic). The orexigenic effect of NPY is mediated by stimulation of hypothalamic Y1R and Y5R receptors in addition to local inhibition of POMC neurons in the ARC. Injection of NPY stimulates food intake, and chronic treatment with NPY results in hyperphagia and increased weight gain. POMC is a precursor for melanocyte-stimulating hormone (α-MSH) which binds to melanocortin-4 receptors (MC4R) in the PVN to suppress food intake. Although CART is an endogenous inhibitor of food intake, its function is rather ambiguous. CART injected into the cerebrospinal fluid suppressed food intake, but an injection of CART directly into the PVN or Arc causes an increase in food intake. This suggests that CART has alternative effects on food intake depending on the site of administration. AgRP stimulates food intake by acting as an antagonist at MC3R and MC4R in the PVN. Other nuclei within the hypothalamus are also implicated in the control of food intake. The LHA contains the orexigenic hormones MCH and orexin. In the VMN,

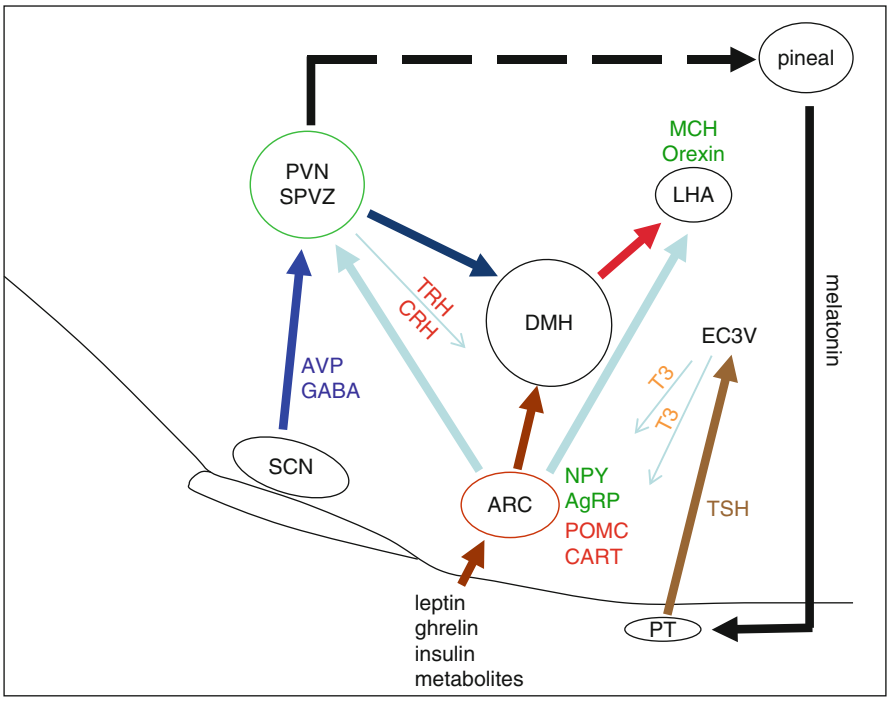

Fig. 19 Major brain centers controlling circadian and homeostatic regulation of food intake and metabolism as depicted in a sagittal plane. The arcuate nucleus (*ARC*) plays a central role in the regulation of hunger and satiety; it has the ability to monitor the peripheral metabolic status through hormones such as ghrelin, insulin, and leptin and through metabolites such as fatty acids and glucose via the blood stream. ARC projects to the dorsomedial nucleus of the hypothalamus (*DMH*), the paraventricular nucleus of the hypothalamus (*PVN*), and lateral hypothalamic area (*LHA*). Neurons in the ARC which coexpress neuropeptide Y (*NPY*) and agouti-related peptide (*AgRP*) stimulate food intake (they are orexigenic), whereas neurons coexpressing pro-opiomelanocortin (*POMC*) and cocaine- and amphetamine-regulated transcript (*CART*) suppress feeding (they are anorexigenic). The LHA contains the orexigenic hormones melanin-concentrating hormone (*MCH*) and orexin. The PVN produces the anorectic thyrotropin-releasing hormone (*TRH*) and corticotropin-releasing hormone (*CRH*). TRH has an important role in the regulation of energy homeostasis not only through effects on thyroid function but also through central effects on feeding behavior, thermogenesis, locomotor activation, and autonomic regulation. The PVN, the LHA, and the DMH receive strong direct and indirect SCN projections and might be involved in the circadian regulation of food intake and energy expenditure. Rhythmic expression of molecular clockwork components has been demonstrated in various brain regions including ARC, DMH, the hypophysial pars tuberalis (*PT*), and the ependymal cell layer of the third ventricle (*EC3V*). The pars tuberalis is under control of the melatonin signal derived from the pineal gland (see also Fig. 14). The PT produces TSH that acts upon the ependymal cell layer of the third ventricle and controls the local T3 concentration

brain-derived neurotrophic factor (BDNF) is highly expressed and suppresses food intake through MC4R signaling. Moreover, the VMH expresses the cannabinoid receptor type 1 (CB1) which mediates orexigenic effects of endocannabinoids on

food intake. Interestingly, hypothalamic endocannabinoids are under partial negative control of leptin. The PVN produces the anorectic thyrotropin-releasing hormone (TRH) and corticotropin-releasing hormone (CRH). TRH has an important role in the regulation of energy homeostasis not only through effects on thyroid function but also through central effects on feeding behavior, thermogenesis, locomotor activation, and autonomic regulation.

The PVN, the LHA, and the DMH receive strong direct and indirect SCN projections and might be involved in the circadian regulation of food intake and energy expenditure. Rhythmic expression of molecular clockwork components has been demonstrated in various brain regions including the Arc, the DMH, and the median eminence of the hypothalamus as well as in the ependymal cell layer of the third ventricle. Mice with a mutation in clock gene *Clock* develop alterations in energy metabolism, hyperphagia, and obesity with accompanying symptoms of the metabolic syndrome. In contrast, mice with a targeted deletion of the clock gene *Bmal1* have a reduced lifespan, display various symptoms of early aging, and show a reduction in body fat. This demonstrates the importance of the molecular clockwork in the control of food intake and metabolism.

Restricted Feeding Provides an Important Zeitgeber

As discussed above, the SCN is the major conductor of circadian physiology. However, recent developments have shown the existence of important pacemakers located outside the SCN. One of these is the so-called food-entrainable oscillator (FEO). This oscillator has been discovered in animals that were subjected to restricted feeding, which means the animals were offered food only for a limited time during their sleep phase. Restricted feeding induces increases in locomotor activity shortly before the time point of food presentation, the so-called food anticipatory activity (FAA), and entrains the molecular clockwork in the liver. FAA is not altered in SCN-lesioned animals, suggesting that the FEO does not involve the SCN. The location of the FEO has not yet been precisely determined. It may be based on a neuronal network comprising various hypothalamic and limbic nuclei. Apparently, the DMH play an important role since FAA is affected by DMH lesions and restricted feeding results in robust oscillations of the Per1 and Per2 genes in the DMH.

Molecular Clocks Are Ticking in Peripheral Organs

Importantly, molecular clockworks are not restricted to the SCN, but are present in a variety of brain regions outside the SCN and peripheral organs (“slave oscillators” or peripheral clocks). With a few exceptions, the components of the molecular clockwork in the SCN, the circadian rhythm generator, and in the peripheral slave oscillators seem to be identical, and, like the SCN, peripheral clocks are

temperature-compensated. Nevertheless, there is an important difference between the SCN and peripheral clocks: as mentioned above, the rhythm generated by the SCN is self-sustained and is maintained for several months in the isolated SCN kept in a culture dish. On the contrary, the rhythms in peripheral tissues are lost after several cycles, which means they dampen. Most probably, this dampening reflects a desynchronization of the cells in the peripheral oscillators rather than a loss of their individual circadian rhythmicity.

Peripheral Clocks Need to Be Synchronized

To maintain proper phase relationships with each other, the slave oscillators in peripheral organs need to be coordinated, and the SCN plays a major role for this coordination since the phase coherence in peripheral clocks is lost in SCN-lesioned animals. The SCN sends timing signals for synchronization of peripheral clocks via multiple pathways: rather direct signals are transmitted through the parasympathetic and sympathetic nervous system and via the neuroendocrine hands of the SCN such as melatonin or glucocorticoids. Moreover, the SCN provides indirect cues to the clocks in the periphery via its impact on the body temperature rhythm and the rest-activity rhythm which in turn drives the feeding rhythms. The feeding-fasting cycles which under normal conditions are in phase with the rest-activity rhythms appear to be dominant synchronizing signals for the peripheral clocks in the liver, kidney, pancreas, and heart. In these organs, the expression profiles of many circadian genes are influenced by the timing of food intake. This entrainment may be mediated by hormones secreted upon feeding or fasting, e.g., cholecystokinin, ghrelin, or leptin; by food metabolites, e.g., glucose, cholesterol, and fatty acids; by postprandial temperature elevations; and by the intracellular redox state ratio. According to current concepts, synchronization of peripheral clocks cannot be accomplished by a single "entrainment" signal, but requires a combination (cocktail) of signals. These cocktails appear to differ from one peripheral clock to the other. Moreover, peripheral clocks can be directly entrained by various external (environmental) stimuli, the most prominent of which is feeding. The molecular clockwork in peripheral tissues controls circadian rhythms in metabolic and physiologic cell/organ function. The importance of molecular clocks is underlined by microarray studies showing that up to 20 % of the genes expressed in peripheral organs (liver, muscle, adipose tissue) are rhythmic, suggesting that a considerable portion of the transcriptome is controlled by the circadian system. The rhythmically expressed genes encode proteins and enzymes involved in biosynthetic and metabolic processes such as lipid metabolism, glycolysis and gluconeogenesis, oxidative phosphorylation, and detoxification pathways. Notably, in many of these pathways, the rate-limiting enzymes are under circadian control. These data indicate a close interrelationship between the circadian system and energy metabolism.

The Molecular Clockwork Influences the Cell Cycle

A growing number of recent studies provide evidence for functional links between the molecular clockwork and cell cycle control (Fig. 20). As shown by microarray studies, key components of cell cycle progression show circadian expression profiles and are directly or indirectly activated or inactivated by clock proteins. Thus, the expression of Wee1, p21, and Myc is regulated by the clock proteins PER1/2, REV-ERBα, and CLOCK/BMAL1. The relationship between circadian and cell cycle processes is further demonstrated in transgenic mice with a deletion of *Per2*: these animals have an increased risk for cancer development.

When the Circadian System Becomes Altered

The most frequent reason for alteration of the circadian system in healthy people is the so-called jet lag. Jet lag occurs in individuals who rapidly travel across a number of time zones, i.e., after long-distance transmeridian (east–west or west–east) flights. Under these conditions, the circadian system becomes out of synchronization with the destination time because the environmental light/dark cycle at the destination is out of the phase the circadian system has been accustomed to. Symptoms of jet lag vary depending on the number of time zones crossed and on individual differences; they include irregular sleep patterns, insomnia, fatigue, mild depression, constipation, or diarrhea. In general, travel from west to east appears more disruptive than from east to west. As shown in experimental animals, the time needed for full entrainment to the new environment varies between the rhythm generator in the SCN and subsidiary clocks in the periphery. While the SCN entrains to the new

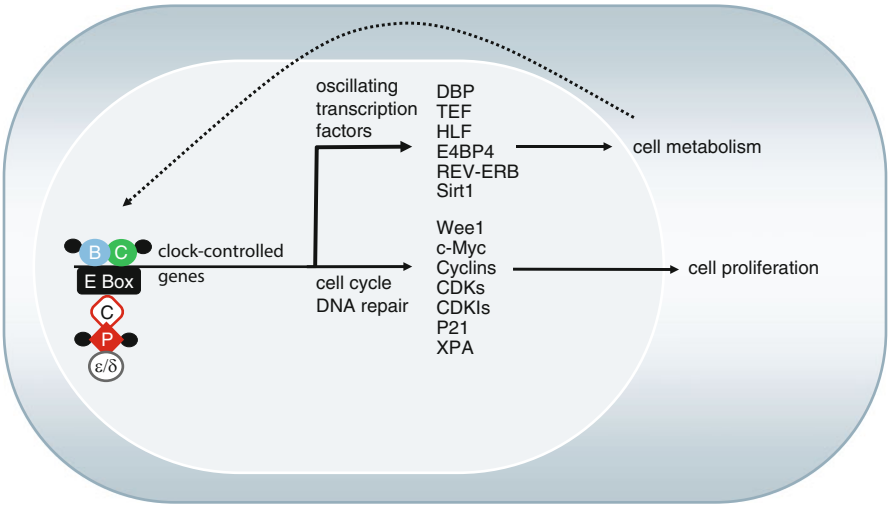

Fig. 20 Interactions between clock genes and genes controlling cell proliferation and metabolism

environment within the first cycle, the subsidiary clock in the liver becomes fully adjusted only after six cycles. The effects of jet lag can be minimized by appropriately timed treatment with bright light (preferably sunlight) and/or melatonin.

Notably, humans show considerable differences in their preferred timing of sleep and activity, yielding a continuum of so-called chronotypes. In a given population, chronotypes range from early (morning) to late (evening) types, the so-called larks and owls. The mechanisms underlying these differences are not yet fully understood; they may be related to clock gene variation or environmental influences. In owls, the sleep timing largely differs between work and free days resulting in a so-called social jetlag and a considerable sleep debt on work days.

Several studies in experimental animals and man have shown that the circadian system is disrupted in numerous diseases. These include the familial advanced sleep phase syndrome (FASPS), the delayed sleep phase syndrome, seasonal affective disorder, uni- and bipolar depression, autism spectrum disorders, Alzheimer's disease, Parkinson's disease, and Huntington's disease. Sleep disturbances in aging people can be partially attributed to age-related reduction in amplitude and phase advance of circadian rhythms. Moreover, the close relationship between circadian and metabolic cycles suggests that the metabolic syndrome is associated with disturbances of the circadian system.

Outlook

Basic research during the last two decades has revolutionized our understanding of the circadian system. A breakthrough has been the identification of molecular clockworks comprising clock genes that interact in transcriptional/translational feedback loops and control the activation or inactivation of clock-controlled genes. Molecular clockworks can be found in virtually all cell types. They form the core of the master clock that in mammals resides in the suprachiasmatic nuclei of the hypothalamus and they are also present in multiple subsidiary clocks in the periphery (e.g., heart, liver, lung, kidney). To maintain proper circadian physiology, the molecular clockworks need to be coordinated and synchronized. Of particular interest appear the mechanisms and pathways through which the master clock coordinates the multiple subsidiary clocks in the periphery. According to current concepts, this synchronization cannot be accomplished by a single "entrainment" signal, but requires a combination (cocktail) of signals. Such cocktails appear to differ from one peripheral clock to the other, and a fascinating perspective of future research is the identification of novel "synchronizing" components and cocktails.

The circadian system influences a plethora of body functions: it drives the rhythm in locomotor activity and is involved in the control of the sleep-wake cycle and body temperature. Most recent investigation has disclosed an extensive cross talk between the circadian system and metabolic systems. Moreover, the circadian system appears to affect the cell cycle. A challenging task for future research will be the clarification of the mechanisms underlying these widespread interactions at the systemic, cellular, and molecular levels.

The circadian system may become altered or disrupted under various conditions. In healthy people, the circadian system is disturbed after rapid travel across a number of time zones, resulting in the so-called jet lag. Notably, disruptions of the circadian system are also observed in numerous diseases. These include the familial advanced sleep phase syndrome, the delayed sleep phase syndrome, seasonal affective disorder, uni- and bipolar depression, autism spectrum disorders, Alzheimer's disease, Parkinson's disease, and Huntington's disease. Sleep disturbances in aging people can be partially attributed to age-related reduction in amplitude and phase advance of circadian rhythms. The circadian system may also affect other pathologies that cluster at specific times of the day, such as stroke, myocardial infection, arrhythmogenicity, and thrombosis.

The breakthroughs in contemporary chronobiology now provide an excellent basis to develop and establish a rational and systematic "chronomedicine" involving a broad range of clinical disciplines from psychiatry to oncology and social medicine.

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