Basic Circadian Timing and Sleep-Wake Regulation

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Abbreviations

5-HT	5-hydroxytryptamine or serotonin
A1	Adenosine type 1 receptor
A2a	Adenosine type 2a receptor
AA-NAT	Arylalkylamine *N*-acetyltransferase
ACh	Acetylcholine
ACTH	Adrenocorticotropic hormone
AMPA	Alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ARC	Arcuate nucleus
BDNF	Brain-derived neurotropic factor
BNST	Bed nucleus of the stria terminalis
cAMP	Cyclic adenosine monophosphate
cGMP	Cyclic guanosine monophosphate
CBT	Core body temperature
ccgs	Clock-controlled genes
CR	Constant routine
CRE	Calcium/cAMP response element
CREB	CAMP response element binding
CRH	Corticotropin-releasing hormone
DMH	Dorsomedial nucleus of the hypothalamus
DMV	Dorsal motor nucleus of the vagus
DRN	Dorsal raphe nuclei
EEG	Electroencephalography
EGF	Epidermal growth factor
EMG	Electromyography
ERKs	Extracellular signal-regulated kinases
EOG	Electrooculography
GABA	Gamma-aminobutyric acid
GCs	Glucocorticoids
GHT	Geniculohypothalamic tract
GLU	Glutamate
HB	Habenula
IL-1β	Interleukin-1 beta
IML	Intermediolateral column
IGL	Intergeniculate leaflet
LC	Locus coeruleus
LS	Lateral septum
LDT	Laterodorsal tegmental nucleus
LHA	Lateral hypothalamus area
LPT	Lateral pontine tegmentum
MPO	Medial preoptic area
MRN	Medial raphe nuclei
MT2	Melatonin type 2 receptor
NMDA	*N*-Methyl-D-aspartate
NO	Nitric oxide
NPY	Neuropeptide Y
NREM	Non-rapid eye movement
ORX	Orexin
PACAP	Pituitary adenylate cyclase-activating peptide
PC	Precoeruleus
PPT	Pedunculopontine tegmental nucleus
Process C	Circadian process of sleep-wake regulation
Process S	Homeostatic process of sleep-wake regulation
PSG	Polysomnography
PVN	Paraventricular nucleus of the hypothalamus
PVT	Paraventricular nucleus of the thalamus
RGT	Retinogeniculate tract
RORE	ROR-specific response elements
REM	Rapid eye movement
RHT	Retinohypothalamic tract
SCG	Superior cervical ganglion
SCN	Suprachiasmatic nucleus
SE	Sleep efficiency
SL	Sleep latency
SLD	Sublaterodorsal nucleus
SPZ	Subparaventricular zone
SWA	Slow-wave activity
TMN	Tuberomammillary nucleus
TNF-α	Tumor necrosis factor alpha
TRH	Thyrotropin-releasing hormone
TSH	Thyroid-stimulating hormone

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S. Chokroverty (ed.), *Sleep Disorders Medicine*, DOI 10.1007/978-1-4939-6578-6_6

TST	Total sleep time
USW	Ultradian sleep-wake cycle
VIP	Vasoactive intestinal polypeptide
VLPO	Ventrolateral preoptic area
vPAG	Ventral periaqueductal gray matter
WASO	Wake after sleep onset
WMZ	Wake maintenance zone
ZT	Zeitgeber

The Circadian Timing System

Origin and Definition of Circadian Rhythms

On Earth, life has evolved under the influence of the cyclic exposure to sunlight and darkness due to the planet's revolution around its axis. As one rotation is achieved in 24 h, most living organisms display rhythmic variations with a similar period for numerous biologic parameters, such as gene and protein production, physiological functions, behaviors, and cognitive processes. It is thought that evolution has led to the development of species that could integrate this rhythmic variation in their functioning allowing them to anticipate environmental changes in order to increase their chances of survival. While the first written description of biologic rhythms was made during the fourth century B.C. by Androsthenes, it is only in 1729 that the French astronomer Jean-Jacques Dortous de Mairan demonstrated that these rhythms are not passive responses to variations of the outside world. By reporting the persistence of daily leaf movements of the sensitive plant *Mimosa pudica* in constant conditions (i.e., constant darkness), he was one of the first to demonstrate their endogenous origin [1].

Today, these intrinsic oscillations, found in humans and in almost all organisms among animals, plants, fungi, and cyanobacteria [2], are known to be driven by the circadian system (from the Latin *circa* and *dies*, meaning "about" and "one day," respectively). In mammals, this system comprises a multitude of oscillatory structures called "clocks" that are found throughout the body and are able to generate circadian rhythms through a hierarchical organization. For a biologic rhythm to be considered as circadian, it has to fulfill the following properties: The rhythm has to be endogenous, meaning that it persists under constant conditions; the rhythm has to be synchronized or entrained by external and internal cues (called *zeitgebers* or ZT from the German, meaning "time-giver"), the most potent being the light/dark cycle; the rhythm has to be temperature-compensated, meaning that a given rhythm remains constant when temperature increases or decreases across a physiological range [3, 4].

Neurolocalization of Circadian Rhythms: The Mammalian Central Clock

In mammals, circadian rhythms are driven by a central or "master" clock enclosed in a well-defined bilateral region of the anterior hypothalamus, namely the suprachiasmatic nuclei (SCN). The first demonstration establishing that the SCNs are essential for the production of circadian rhythms was published in 1972 in two independent studies, in which a bilateral lesion of the SCNs led to the loss of the cortisol secretion rhythm [5], as well as the rest-activity and drinking behavior rhythms [6]. A later study showed that a transplantation of fetal SCN was able to restore behavioral rhythmicity in hamsters bearing SCN lesions [7]. Notably, the period of the restored rhythm was the period expressed by the SCN of the donor. With this elegant demonstration, Ralph and collaborator demonstrated that the SCNs are the primary structures that generate internal circadian rhythmicity.

Anatomically, the SCNs are two nuclei located in the anterior hypothalamus, on top of the optic chiasm, bilateral to the third ventricle [8]. In humans, each SCN contains around 50,000 neurons for an approximate volume of 0.25 mm^3 [9–11] and can be subdivided roughly into "core" and "shell" regions, also described in most mammalian species [8, 12]. While the ventrolateral "core" neurons largely express vasoactive intestinal polypeptide (VIP), the dorsomedial "shell" neurons are rich in arginine vasopressin (AVP), and both neuropeptides co-localize with γ-aminobutyric acid (GABA) and sometimes with glutamate [12]. "Core" neurons primarily receive photic information from the retinohypothalamic tract (RHT) and densely project to the shell. "Core" neurons appear to be essential in the maintenance of internal coupling and the production of a coherent output signal from the SCNs [13]. Both "core" and "shell" regions [14] project mainly to local structures surrounding the SCNs [14, 15] (Fig. 6.1). Numerous other neurotransmitters and neuropeptides have been detected in the SCNs, but their presence and exact localization are species-specific, suggesting that the SCNs organization is more complex than the core-shell subdivision model [16, 17]. Of note, in humans, neurotensin-containing neurons are present in a larger population than that described in monkeys and all other species [17]. Whereas their role is still poorly defined, a loss of neurotensin-containing neurons has been reported in patients suffering from dementia [18, 19] and correlated with dampening in circadian amplitude such that of the core body temperature rhythm (or CBT) [19].

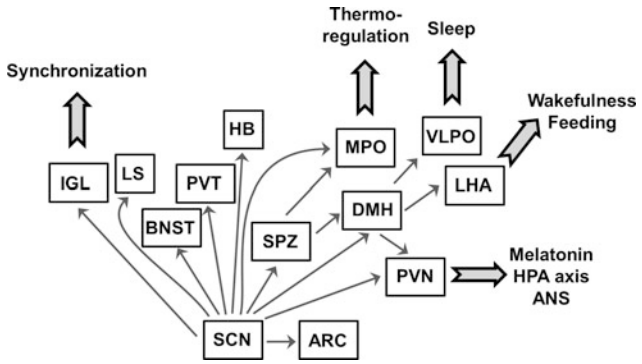

Fig. 6.1 Efferent neural projections from the SCN to different brains regions and their involvement in the regulation of physiological functions. *ARC* arcuate nucleus; *BNST* bed nucleus of the stria terminalis; *DMH* dorsomedial hypothalamus; *HB* habenula; *IGL* intergeniculate leaflet; *LS* lateral septum; *LHA* lateral hypothalamus area; *MPO* medial preoptic area; *PVN* paraventricular nucleus of the hypothalamus; *PVT* paraventricular nucleus of the thalamus; *SCN* suprachiasmatic nuclei; *SPZ* subparaventricular zone; *VLPO* ventrolateral preoptic area

Genesis of Circadian Rhythms: The Mammalian Molecular Clockwork

The molecular mechanisms responsible for the genesis of endogenous circadian rhythms have started to be unfolded in the 1990s. To our knowledge, the first mammalian circadian molecular oscillator model was proposed in 2000 [20], in which the self-sustained rhythmicity is generated by positive and negative components that form feedback core loops with a period of about 24 h (Fig. 6.2; see Table 6.1 for full official/alternative names of the different clock components). Initially, the model was based on reports of behavioral rhythms changes observed in transgenic mice for different "clock" genes. Since 2000, it has been progressively refined with the addition of new components and the development of new transgenic mouse models [3, 21]. Notably, the basic autoregulated feedback core loops have been preserved through evolution and their timing is similar in nocturnal and diurnal species, including humans (for review, see [3]). CLOCK and BMAL1 (two PAS domain helix-loop-helix proteins) represent the positive limb of the loop. In the cytoplasm, CLOCK and BMAL1 translocate to the nucleus where they act as transcription factors on genes containing an E-box (5′-CACGTG-3′) or an E′-box (5′-CACGTT-3′) on their promoter. The genes constituting the negative limb of the loop, namely *Per1/2/3* and *Cry1/2*, contain E-boxes, and their transcription is thus activated by CLOCK/BMAL1. Their protein products, PER and CRY, heterodimerize and bind to CK1ε/δ forming a complex that allows the phosphorylation of PER and CRY. This leads to the translocation of the complex to the nucleus where it accumulates to eventually repress the transcription of several genes, including *Per* and *Cry*. This inhibitory mechanism would be the result of the deacetylation of histones 3/4 following the recruitment of a PTB-associated splicing factor/SIN3-histone deacetylase complex (PSF/Sin3-HDAC complex) by PER/CRY [22].

In addition to this main feedback core loop, interconnected secondary loops and transcription factors have been described, which add supplementary layers of control allowing to fine-tune regulations of the circadian rhythmicity. One of these loops involves the CLOCK/BMAL1 complex, which controls its own production by a regulation of the transcription of other genes containing an E-box, namely *Rev-Erbα/β* and *Rorα/β/γ* (Fig. 6.2). Through a competitive binding on ROR-specific response elements (RORE) present on the *Bmal1* promoter, REV-ERB and ROR proteins can inhibit and activate *Bmal1* transcription, respectively, leading to their cyclic production. Despite this important role, REV-ERB and ROR are not essential to sustain oscillations within the SCN [23]. E-boxes can also be targeted by other transcription factors, such as DEC1/2, which both act to reduce E-box-mediated transcription [24]. Some other second accessory feedback loops involve transcription factors from the proline and acidic amino acid-rich basic leucine zipper family, which can bind to D-boxes (5′-TTATG[T/C]AA-3′) (e.g., DBP and TEF; see Table 6.1) [3].

Transcription factors and transcriptional regulation play a crucial role in the generation of circadian rhythms, but other mechanisms and molecules such as posttranslational modifications, microRNAs, and RNA-binding proteins are also considered important regulatory elements [25, 26]. For instance, the process of elimination of PER and CRY follows a precise timing that is required to end the repression phase and start a new cycle of transcription. Once PER and CRY are targeted by CK1ε/δ and AMPK, respectively, they are polyubiquitylated by β-TRCP1 and FBXL3 (nucleus) or FBXL21 (cytoplasm and nucleus), respectively (proteins that are part of an ubiquitin protein ligase complex, named SKP1-cullin-F-box or SCF ubiquitin ligase complex), which are then degraded by the 26S proteasome (Fig. 6.2). These posttranslational mechanisms allow a control of the stability/degradation rate of the PER and CRY proteins which is crucial to precisely set the period of the central clock [3, 27, 28].

While half of all mammalian genes are expressed rhythmically in at least one tissue [29], it appears that only a small number of genes are truly essential in the genesis of circadian rhythms. All the other rhythmic genes are called clock-controlled genes (ccgs) because they are not involved in the different loops but are regulated by them [30]. Ccgs are considered as the molecular output of the circadian clock. They represent the connection between the feedback core loop and a multitude of cellular and physiological functions, including SCN functions. For example, in the SCN, some of the ccgs, such as the *Avp* gene, are directly under the rhythmic control of CLOCK/BMAL1 via E-boxes [31] and *Avp* is known to be implicated in the circadian regulation of drinking behavior [32]. The rhythmic firing rate of SCN neurons is achieved via the

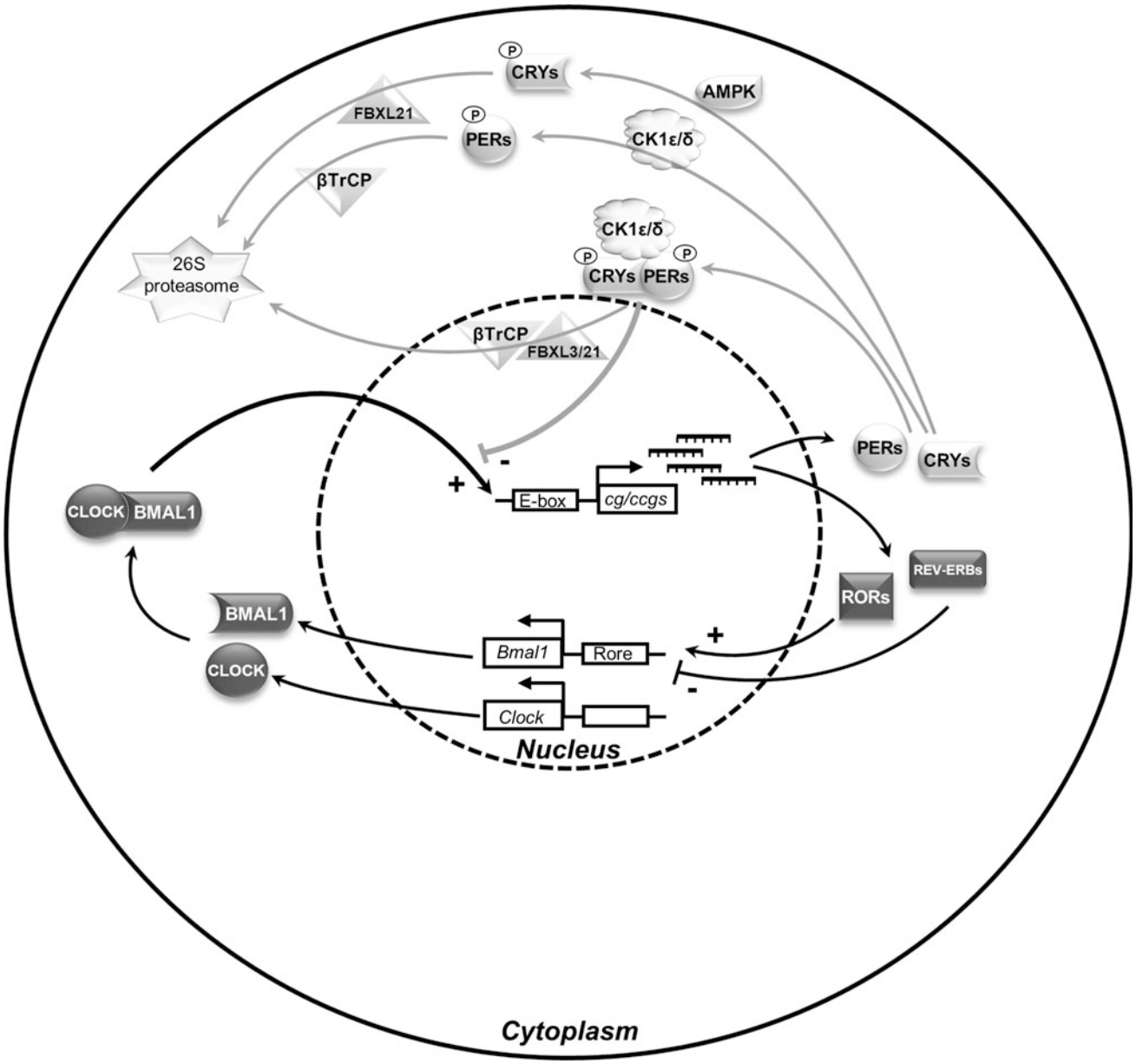

Fig. 6.2 Molecular model of the mammalian cell autonomous oscillator within a SCN cell as described in the text. Genes are represented in *italic* and proteins in CAPITAL. Nomenclature for each element is given in Table 6.1. CLOCK/BMAL1 activates transcription of genes containing an E-Box, including *Per* and *Cry*. PER/CRY, once translated into proteins, inhibit their own transcription. The other actors allow to fine-tune regulations of circadian rhythmicity. *CG* clock gene; *CCGs* clock-controlled genes; *P* phosphate

cyclic regulation of membrane potassium channels, and it is considered as an output of the central clock probably implicating ccgs [33, 34].

Organization of the Circadian System: A Hierarchical Multioscillatory System

For decades, SCN neurons were thought to be the only cells capable of generating self-sustained circadian oscillations explaining why the SCN has been considered as the "master" clock for so long. Today, we know that almost every cell of the body can express the main clock genes in a rhythmic fashion [35], and thus, many brain regions [36–38] and non-neuronal tissues [35, 39–41] can be considered as autonomous clocks, known as peripheral clocks. However, without environmental cues (e.g., in vitro), the oscillations seen in peripheral clocks usually dampen after few days due to a weak coupling between the cells constituting those clocks [42, 43], whereas the SCN can self-sustain

Table 6.1 Non-exhaustive list of core clock genes and associated circadian genes mentioned in the chapter

Gene	Alternative name	Full name
PER1	*PERIOD1*	Period circadian protein homolog 1
PER2	*PERIOD2*	Period circadian protein homolog 2
PER3	*PERIOD3*	Period circadian protein homolog 3
CRY1	–	Cryptochrome 1
CRY2	–	Cryptochrome 2
BMAL1	*ARNTL*; *MOP3*	Brain and muscle aryl hydrocarbon receptor nuclear translocator-like protein 1
CLOCK	–	Circadian locomotor output cycle kaput
NPAS2	*MOP4*	Neuronal PAS domain-containing protein 2
RORα	*RORa*, *NR1F1*	Retinoic acid-related orphan receptor alpha
RORβ	*RORb*, *NR1F2*	Retinoic acid-related orphan receptor beta
RORγ	*RORc*, *NR1F2*	Retinoic acid-related orphan receptor gamma
REV-ERBα	*NR1D1*	Reverse viral erythroblastosis oncogene product alpha
REV-ERBβ	*NR1D2*	Reverse viral erythroblastosis oncogene product beta
CK1δ	*CSNK1d*	Casein kinase I delta
CK1ε	*CSNK1e*	Casein kinase I epsilon
AMPK	–	AMP-activated protein kinase
DBP	–	D-box binding protein
TEF	–	Thyrotroph embryonic factor
B-TRCP1	*BTRC*	Beta-transducin repeat containing protein 1
FBXL3	–	F-box and leucine-rich repeat protein 3
FBXL21	–	F-box and leucine-rich repeat protein 21
DEC1	*BHLHE40*	Differentiated embryo chondrocyte protein 1
DEC2	*BHLHE41*	Differentiated embryo chondrocyte protein 2
AVP	*ADH*	Arginine vasopressin

synchronized rhythms for weeks due to their specific neuronal network. The unique properties of SCN cells are due to their tight coupling elicited through synaptic connections and gap junctions that allow them to remain synchronized with each other even in the absence of a ZT [12].

In peripheral clocks, the molecular mechanisms responsible for the generation of circadian rhythms are similar to those described for the central clock despite the fact that some distinct genetic factors have been described [44, 45]. For instance, in some brain regions including the striatum and the hippocampus, CLOCK is substituted by its analogue NPAS2, while in the liver, both CLOCK and NPAS2 are expressed, but NPAS2 cannot maintain circadian oscillation when CLOCK is missing. Another example defines RORγ as being expressed specifically in peripheral tissues, but with a similar role to this attributed to RORα/β.

In a physiological point of view, these brain- and non-brain tissue-specific clocks, and ultimately, brain and organ functions are likely to be coordinated by the local circadian molecular machinery associated with the integration of systemic synchronizing factors. This dual control leads to the expression of robust circadian oscillations in peripheral clocks. It appears that most of these synchronizing factors primarily originate from the SCNs, although feeding-fasting cycles and temperature variations are known to entrain peripheral clocks [46, 47]. Based on this dual control, the current model of circadian organization in mammals postulates a hierarchical system in which the central clock in the SCNs controls a network of subordinate peripheral clocks throughout the brain and periphery [48]. Despite this apparent organization, it has been demonstrated that some peripheral structures can drive local physiological rhythms independently of the SCNs, such as the retina [41], the olfactory bulb [49], and the adrenal gland [50]. In addition, a line of evidence has shown the existence of two SCN-independent oscillators, namely the food-entrainable oscillator and the methamphetamine-sensitive circadian oscillator [51–57]. These two oscillators are known to control behavioral and hormonal rhythms even in SCN-lesioned animals or in the absence of clock genes, but neither the site nor the mechanisms underlying their functioning have been yet elucidated.

Communication Within the Circadian System: The Neural and Endocrine Pathways

We previously mentioned that neurons from the SCN project mainly to brain regions restricted to hypothalamic nuclei, with the main outputs directed to the subparaventricular zone (SPZ) and the dorsomedial nucleus of the hypothalamus (DMH) (Fig. 6.1) [58]. By projecting to other brain structures, the SPZ and DMH, and the other nuclei can control the rhythmicity of peripheral clocks and their outputs. One of these pathways connects the SCN to the ventrolateral preoptic area (VLPO) via the dorsal SPZ, which is known to control the circadian sleep-wake cycle [59]. Another pathway links the SCN to the medial preoptic area (MPO) through the ventral SPZ regulating the CBT rhythm [59]. In addition, other neuronal pathways areas of particular importance convey information outside of the brain to peripheral tissues. Indeed, using transneuronal retrograde tracers, distinct polysynaptic sympathetic and parasympathetic routes have been identified emerging from the SCN to end up in the liver, kidney, pancreas, bladder, heart, adrenal cortex, spleen, and brown adipose tissues [60–63]. For instance, connections emerging from the SCN reach the adrenal gland via the paraventricular nucleus of the hypothalamus (PVN) and the intermediolateral column (IML) (i.e., sympathetic pathway) and control the rhythm of sensitivity to adrenocorticotropic hormone (ACTH) [64, 65]. Sympathetic projections also control the rhythmicity of melatonin synthesis, a fundamental circadian endocrine factor. To do so, neurons from the IML project to the pineal gland via the superior cervical ganglia (SCG) and regulate in a rhythmic fashion the production of the rate-limiting enzyme arylalkylamine *N*-acetyltransferase (AA-NAT), implicated in melatonin synthesis [66, 67]. The parasympathetic nervous system also conveys rhythmic information via fibers originating in the SCN, which then reach the PVN and the dorsal motor nucleus of the vagus (DMV) allowing for instance a regulation of peripheral clocks found in white adipose tissue and the liver [68, 69]. It seems that through all these innervations, the SCN has the potential to orchestrate the circadian rhythmicity of peripheral oscillators and their metabolic and physiological functions throughout the entire body.

The SCN can also synchronize peripheral oscillators through a rhythmic control of numerous actors of the endocrine system. Among them, few hormones have been well described as regulators of the circadian system. As we previously mentioned, melatonin secretion is regulated by the SCN, and the resulting rhythm of melatonin is known to upregulate some actors of the immune system and to control several seasonal functions [70, 71]. In addition, melatonin would specifically facilitate sleep in humans [70]. Glucocorticoids or GCs (i.e., corticosterone in rats and mice; cortisol in humans) represent another class of hormones that seems essential for the regulation of peripheral clocks. Rhythmic GCs synthesis and secretion are under control of the central clock through direct and indirect projections reaching the PVN. In the PVN, parvocellular neurosecretory cells release corticotropin-releasing hormone (CRH) that controls the release of ACTH from the pituitary gland, itself controlling GCs release by the adrenal glands [50, 72]. The rhythm of GCs is known to regulate the synchronization of many peripheral clocks at both behavioral and molecular levels [45, 72]. The SCN also controls, through the PVN, the release of AVP and thyrotropin-releasing hormone (TRH), the latter stimulating the release of thyroid-stimulating hormone (TSH) and prolactin. Other hormones such as epinephrine and norepinephrine [73], leptin, glucose, and insulin [74] express a circadian rhythm and could constitute endocrine signals by which the central clock synchronizes peripheral oscillators, but further studies are needed to clarify their respective roles.

Synchronization of the Circadian System: External and Internal Factors

In animals, including humans, the endogenous period (or internal day length) of most circadian rhythms is close to, but not exactly, 24 h [75, 76]. To be in phase with the external world, the circadian system is continuously resynchronized to the 24-h terrestrial day through a mechanism called "synchronization" or "entrainment." As a relay between the external environment and the internal functions of the body, the SCN represents the primary structure implicated in this mechanism. Among external factors that influence the circadian system, the 24-h light/dark cycle is the most powerful and the most studied, but it has been demonstrated that variations in environmental temperature or food availability can act as synchronizing factors for the central clock [46, 77, 78]. Notably, a wide range of internal factors that convey physiological information to the central clock are integrated by the SCN in conjunction with light cues and thus participate in the synchronization of the SCN. Some of those internal factors will be described later in this chapter. Of note, in constant conditions, the period of time when nocturnal species are awake is considered as the "subjective" night, while it is named "subjective" day when they are asleep and vice versa for diurnal species.

The Photic Factor: Molecular and Behavioral Effect

In mammals, it has been known for decades that light information is received by image-forming rods and cones' photoreceptors located in the retina, which is then transmitted to the cerebral regions involved in visual processing. While rods and cones are also implicated in the detection of light information that is conveyed to the SCN, a subset of retinal ganglion cells expressing the photopigment

melanopsin (Fig. 6.3) has been described as fundamental for the SCN to be synchronized to the light/dark cycle [79]. Melanopsin cells are present in 0.2–2.5 % of the total number of retinal ganglion cells [79–81] and their maximal photosensitivity occurs in the blue-green light spectrum (~470 nm) [82]. Along with rods and cones, melanopsin cells convey photic information using a direct pathway, the RHT (Fig. 6.3) that has been described in mammals including humans [83–86]. The RHT innervates the ventrolateral "core" region of the SCN and releases glutamate and pituitary adenylate cyclase-activating peptide (PACAP). Moreover, an indirect pathway has been described that connects melanopsin cells to the ventral part of the SCN via the intergeniculate leaflet (IGL), which releases GABA and neuropeptide Y (NPY, Fig. 6.3) [87, 88]. Of note, the IGL is a thalamic structure implicated in the integration of photic and non-photic signals [87–89].

Glutamate released by RHT neuron terminals leads to the activation of multiple signaling pathways in the SCN following activation of *N*-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors [90]. This activation leads to a calcium influx into SCN neurons, which stimulates the synthesis of nitric oxide (NO) [91] known to act differentially depending on the time of day. During the early night, NO induces an activation of ryanodine receptors, which is followed by Ca^{2+} release from the endoplasmic reticulum [92]. During the late night, NO activates guanylate cyclase that stimulates the synthesis of cyclic guanosine monophosphate (cGMP) [92]. A third pathway, implicating cyclic adenosine monophosphate (cAMP) is effective throughout the night [93] and together these three pathways lead to the activation of the protein kinase A and extracellular signal-regulated kinases (ERKs), which phosphorylate cAMP response element binding (CREB) protein [94–96]. In turn, CREB activates *Per1* and *Per2* transcription in the SCN in response to its binding to the cAMP response element (CRE) present on *Per* gene promoters [12, 95–97]. Using alteration of *Per1/2* transcription [98, 99] and *Per1/2* mutant mice [100], these two genes have been attributed a causal role in the mediation of light-induced synchronization of the SCN. Both genes present temporal and spatial differences in their response to the photic factor [101–104]: *Per1* transcription is quickly induced throughout the night in ventral SCN cells; *Per2* expression is induced in the whole SCN, quickly in the early night and slowly or not at all in the late night. Of note, the expression of several other genes and clock genes (i.e., *Bmal1*, *Cry2*, *Rev-erbα*, *Rorβ*, and *c-fos*) is also regulated by light, but their role is less clear [101, 105–110].

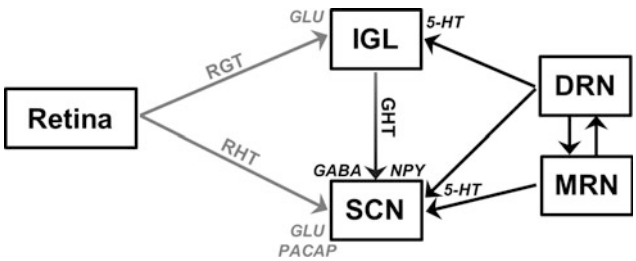

Fig. 6.3 Main afferent neural projections to the SCN, RHT and RGT convey photic cues from the retina to the SCN and the IGL, respectively (*gray arrows*). Non-photic information is conveyed from raphe nuclei (DRN and MRN) to the SCN and the IGL (*black arrows*). The IGL conveys both photic and non-photic cues to the SCN (*black/gray arrow*). Neuropeptides and neurotransmitters are indicated in *italic*. *5-HT* serotonin; *DRN* dorsal raphe nucleus; *IGL* intergeniculate leaflet; *GABA* γ-aminobutyric acid; *GHT* geniculohypothalamic tract; *GLU* glutamate; *MRN* median raphe nucleus; *NPY* neuropeptide Y; *PACAP* pituitary adenylate cyclase-activating peptide; *RGT* retinogeniculate tract; *RHT* retinohypothalamic tract; *SCN* suprachiasmatic nuclei

Thus, from the retina, photic information induces a daily resetting of the central clock by a modulation of the molecular clock leading to changes of the circadian phase of SCN neurons. As SCN neurons form a tightly coupled network, the entire SCN quickly adjusts to a shift of the light/dark cycle. The phase of the SCN can be delayed or advanced by several hours each day in most mammalian species, according to the time of day when the retina receives light [89, 111]. The central clock can then relay photic information to peripheral oscillators through neuronal connections or endocrine signals as previously described, leading to phase adjustments of the circadian rhythms of these oscillators as well as those of their outputs (e.g., locomotor activity, feeding timing, and CBT). For instance, to assess the effects of light on the rhythm of locomotor activity, it is possible to apply discrete light pulses at different time points in animals or humans studied in constant conditions (e.g., constant darkness in rodents and constant routine or CR in humans). Using this method, a phase response curve to light can be defined, which expresses the magnitude of the induced phase shift as a function of the time of light exposure (Fig. 6.4). In both nocturnal and diurnal species, including humans, the phase response curve to light displays a similar pattern with the larger light-induced phase shifts occurring mainly during or close to the subjective night (i.e., active and resting period in nocturnal and diurnal species, respectively) [89, 101, 112–116].

In humans, light exposure in early morning (i.e., after CBT reached its minimal value) is known to phase advance the circadian system [114], while light exposure at night (i.e., before the CBT minimum) results in phase delay of the circadian system. Light intensity [117–119], wavelength [120, 121], and prior light exposure [122] were shown to be important determinants of light-induced phase shifts of the circadian system.

Using a simulated night shift experiment, we recently demonstrated the ability of light to entrain peripheral clocks in humans, through synchronization of the central clock [123].

The Non-photic Factors: Focus on Melatonin, Activity, Sleep and the Serotonergic System

Besides light, many factors can modify the rhythmicity of the circadian system at both molecular and behavioral levels. The most studied non-photic factors cited in this chapter and their effects on clock gene expression in the SCN and the rest-activity cycle are summarized in Table 6.2. This chapter will focus on two categories of non-photic factors, melatonin and factors related to activity, sleep, and the serotonergic system. In animals, food intake represents another well-studied non-photic factor known to synchronize the circadian system. As there have been excellent reviews describing them [55–57], it will not be further discussed here. Many other factors that influence the central clock and/or peripheral clocks have been described (e.g., dark pulses, temperature, GCs, and drug of abuse). Most of these studies have been conducted in nocturnal species and the few results obtained with diurnal species highlight differences between these two types of species that could be relevant to human chronobiology, considering the diurnal nature of human life.

To date, melatonin has been the most studied non-photic synchronizer in humans (for a review, see [124]), and its phase-shifting effects have also been described in several nocturnal and diurnal mammals (Table 6.2). In humans, exogenous melatonin induces phase advances when administered in the late afternoon/evening and phase delays when given in the morning [125, 126]. Similar results have been reported in the evening in nocturnal and diurnal rodents [127, 128]. These effects would be dependent on the

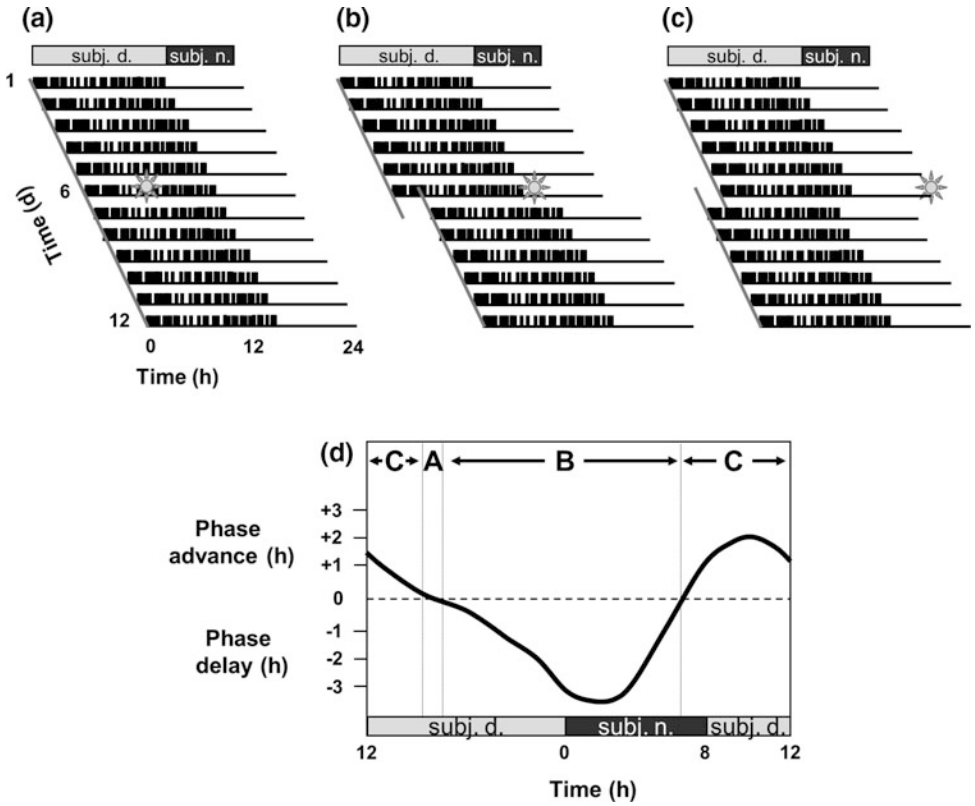

Fig. 6.4 Schematic illustration of light-induced phase shifts of the rest-activity cycle in diurnal humans. Schematic actograms (A, B, C) illustrate the rest-activity rhythm of diurnal humans kept under constant conditions (e.g., constant routine). An actogram is a graph that plots activity levels. In each actogram, twelve successive days are represented from top to bottom, one row corresponding to one day (24-h period). The subjective days (16 h long) and nights (8 h long) are indicated by the *gray* and *black bars*, respectively, at the top of each actogram. In constant conditions, humans express their endogenous period (usually longer than 24 h) explaining why, for a given day, the onset of activity occurs later than the previous day. On the 6th day of recording, a light pulse, represented on the actograms by a *gray* "*sun*," is applied in the middle of the subjective day (*A*), the beginning of the subjective night (*B*), or the end of the subjective night (*C*). In response, the rest-activity rhythm is either not significantly shifted (*A*), phase delayed (*B*), or phase advanced (*C*). Thus, each discrete pulse of light, given at a specific time, leads to various magnitude and direction of shifts. (*D*) represents the phase response curve of the resetting effect of light with by convention phase advance and delays expressed as positive and negative phase shifts, respectively. *subj. n.* subjective night; *subj. d.* subjective day

activation of melatonin type 2 receptor (MT2), which have been found in the SCN of mammals including humans [129–131]. However, *Per* expression is not modified in response to melatonin injections in nocturnal rats [101].

Another well-studied category of non-photic factor comprises behavioral and pharmacological stimuli that are related to levels of activity, sleep deprivation, and the serotonergic system through direct (implicating serotonin or 5-hydroxytryptamine (5-HT) and 5-HT agonists) and indirect (via the IGL and implicating GABA and NPY) connections between the dorsal and median raphe nuclei (DRN and MRN) and the SCN (Fig. 6.3) [132–136]. In nocturnal species, these factors are mainly effective when applied during the middle of the subjective day and they lead to a phase advance of locomotor activity rhythm and a decrease in the expression of *Per1* and/or *Per2* in the SCN [137–142]. Results obtained from diurnal species are limited and provide different results (Table 6.2) [143–148] from those observed in nocturnal species, underlying the differences between these species in terms of sensitivity and responses to non-photic factors. For instance, sustained physical exercise in humans results in a phase delay of the human circadian system when performed at night or in the morning, and a phase advance when exercise is performed in the evening [146, 149–151]. Despite these resetting effects, exercise has been shown to have no effect on the endogenous circadian period of rhythmic parameters in humans [151, 152]. These observations raise an important issue for the development of pharmacological and therapeutic tools that act on the circadian system because they are usually developed and tested in nocturnal species for possible applications to humans.

Of note, this category of activity/serotonin factors highlights the tight connections between the sleep-wake cycle and the circadian system. First, the sleep-wake cycle is closely linked to the activity of 5-HT neurons. For instance, the activity of 5-HT neurons in the cat dorsal raphe is regular and slow during waking episodes [153]. This activity decreases and loses its rhythmicity at the beginning of a sleep episode, until the complete disappearance at the onset of rapid eye movement (REM) sleep episode. Second, 5-HT release in the SCN region occurs during the waking period of nocturnal and diurnal animals and is correlated with the electrical activity of 5-HT neurons [110, 143, 154, 155]. In addition, hyperactivity is known to induce a release of 5-HT into the SCN through fibers originating from the MRN [155], and lesions of 5-HT fibers can block the behavioral phase advances normally present in response to hyperactivity [156]. Third, an induced transient 5-HT deficiency in rats leads to a temporary loss of the circadian rhythms in

Table 6.2 Main behavioral and molecular effects of non-photic factors

Type of factor	Nocturnal species			Diurnal species		
	Timing	Rest-activity rhythm	*Per1/2* expression	Timing	Rest-activity rhythm	*Per1/2* expression
Melatonin	Evening	Phase advance [127]	No effect [101]	*Rodents*: Evening	Phase advance [128]	?
				Humans: Late afternoon/Evening	Phase advance [125, 126]	?
				Humans: Morning	Phase delay [125, 126]	?
Hyperactivity or sustained activity	Midday	Phase advance [137]	Decrease [138]	*Rodents*: Early night	Phase advance [144]	?
				Humans: Evening	Phase advance [146, 149–151]	?
				Humans: Night/morning	Phase delay [146, 149–151]	?
5-HT	Midday	Phase advance [110, 139]	Decrease [139]	*Rodents*: Night	Phase advance [143]	No effect [143]
NPY	Midday	Phase advance [140]	Decrease [141]	?	?	?
GABA	Midday	Phase advance [142, 287]	Decrease [147]	*Rodents*: Midday	Phase delay [147]	Decrease *Per2* [148]

locomotor activity and sleep-wake cycle [157]. Taken together, these results suggest that 5-HT not only promotes wake and inhibits REM sleep [158], but also participates in the circadian regulation of sleep and wake behaviors [157].

The Circadian System in Humans: Specificities and Methods of Investigation

Most of the cellular and molecular data presented in this chapter have been obtained using animal models rather than human subjects, mostly due to the lack of available techniques to measure the intrinsic molecular or electrical activity of the human SCN in vivo and the obvious limited possibilities of human sampling. Nevertheless, laboratories working on human chronobiology typically use robust peripheral output signals primarily regulated by the SCN, thus reflecting the circadian functioning of the central clock. These so-called central circadian markers are derived from physiological measures such as CBT, sleep propensity, or hormone levels such as melatonin and cortisol. Their rhythms are usually defined by their phase, amplitude, and period. The phase represents the time of day when a rhythmic parameter reaches its maximal value (i.e., acrophase or crest time; used for melatonin and cortisol) or its minimal value (i.e., bathyphase or nadir time; used for CBT). The amplitude and period of circadian signals are less commonly reported, though they also provide important information on the state of synchrony/desynchrony between the SCN, peripheral oscillators, and the 24-h environment. In individuals living on regular conditions (entrained to a day-oriented schedule), CBT rhythm typically peaks before habitual bedtime and reaches its minimum values in the second half of the night [159]. Sleep propensity is inversely related to CBT [160]. Melatonin levels start increasing in the evening, reach a peak in the middle of the night, and decrease in the morning with undetectable values occurring during the day [123, 159]. Cortisol secretion peaks just after awakening and slowly decreases through the day to reach its nadir early at night [123]. With the relatively recent discovery of peripheral clocks, markers of those clocks such as clock gene expression (i.e., peripheral markers) have started to be measured in humans as an accessible alternative to central markers. Moreover, they represent a valuable tool to assess the relative synchronization of the different parts of the circadian system. To date, clock gene expression in peripheral oscillators has been measured in different human tissues, including peripheral blood mononuclear cells [159, 161, 162], skin cells [163–165], adipose tissue [166, 167], oral mucosa [163], bone marrow [168], hair follicles [169], and more recently *postmortem* brain regions [38, 170, 171]. Results from these studies indicate that the molecular mechanisms underlying the generation of circadian rhythms are similar in humans and other mammalian species.

In humans, circadian markers can be measured in ambulatory conditions, but they are likely to be masked by behaviors and external stimuli. Negative masking is a process reducing the expression of a circadian pattern [172]. For instance, light exposure at night inhibits the release of melatonin [173]. In contrast, positive masking reflects the expression of a variable in addition to its circadian pattern. In diurnal species, light exposure at night induces a bout of locomotor activity at a time when animals are supposed to sleep [173]. Transient fluctuations in cortisol induced by stress are often high enough to mask the cortisol rhythm [174, 175]. Exercise and feeding have also been described as masking factors that increase the CBT [175, 176]. To precisely describe the circadian profile of a given circadian marker in humans, rigorous protocols need to be used to "unmask" it. Specifically to measure the circadian phase and amplitude, physiological signals have to be measured for at least one complete cycle ($\geq$ 24 h) under constant conditions, such that the influence of the external and internal masking factors (e.g., the sleep-wake cycle, light, food intake, and activity levels) is minimized and/or distributed throughout the day. The most commonly used protocol in human chronobiology, namely the CR (constant routine) [175, 177, 178], is based on this idea and takes place in a laboratory environment of constant dim lighting and ambient temperature. In addition, subjects are requested to stay awake for at least 24 h (up to 72 h), usually in a semirecumbent position to control the level of activity, and meals are replaced by hourly snacks that provide all the essential nutriments needed in a non-circadian rhythmic fashion. Many variations of the CR are in use, with the goal to minimize these masking effects [175]. One of the main issues of the CR is the absence of sleep, especially with protocol >24 h that are known to induce sleep deprivation and negatively affect alertness and performance. As an alternative, a CR protocol with sleep (i.e., constant posture protocol) has been proposed, but by introducing periodic changes in the sleep-wake cycle, parameters such as CBT are affected. Thus, the choice of one or another variant of the CR needs to be based on the type of parameters of interest for a given study. While the CR and its alternatives are very useful to assess the phase and amplitude of central and peripheral circadian markers, other protocols have been developed that focus on other properties of the circadian system. The forced desynchrony protocol induces an internal desynchronization, which is forced by scheduling subjects on sleep-wake regimens that are very different from the classical 24-h rhythm, thus being outside of the range of entrainment of the circadian system (e.g., 28-h day, 20-h day, and 11-h day) [179]. This means that the circadian system cannot be synchronized by the sleep schedule and

instead expresses its own endogenous period. Thus, the phase relationship between the imposed sleep-wake cycle and the circadian system is lost, meaning that the sleep-wake cycle occurs at a variety of circadian phases. This protocol has been very useful to establish the endogenous circadian period of the human central clock and has been used primarily to evaluate circadian and homeostatic influences on observable processes such as sleep, sleep structure, electroencephalographic (EEG) power density in different sleep stages, cognitive performances, mood, and hormonal rhythms (see [179] for references). Other protocols, called multiple naps or ultradian sleep-wake cycle (USW) procedures, have been developed [180–183]. They can be considered as extreme examples of the forced desynchrony protocol. Indeed, in those protocols, the duration of one cycle is usually very short (90 or 120 min) with a wake-sleep ratio that can be of 60/30 min or 60/60 min (the later minimizing the observable sleep restriction). In some extreme cases, the ratio can drop to 15/5 min [184, 185] or 13/7 min [186]. While those protocols do not allow the study of the homeostatic contribution on observable processes, they have the advantage to make possible the examination of the sleep propensity at multiple circadian phases due to the numerous transitions between lights on and lights out.

Circadian Regulation of the Sleep-Wake Cycle

The Sleep-Wake Cycle

As opposed to wakefulness during which functions for survival such as feeding and breeding activities are possible, sleep is a reversible vigilance state characterized by a reduction in voluntary motor functions, sensory perception, and responsiveness and is associated with immobility in a species-specific position. Of note, submitted to the same 24-h light/dark environment, a variety of sleep-wake schedules and sleep durations are observed across species. While some species adopt a diurnal or nocturnal behavior, others are essentially active around dawn and dusk and thus adopt a bimodal behavior [187]. Some species sleep for more than 20 h daily (e.g., cat), while others sleep only for 3 h (e.g., horse) [188]. In humans, sleep is consolidated in a period of approximately 8 h at night, making it one of the most evident behaviors under circadian regulation. It is also interesting to recognize that the sleep-wake cycle can indirectly regulate the circadian system through cyclic exposure to light and darkness. This is especially important in modern society since the development of artificial lighting provides accessible brighter light at night, facilitating nocturnal activities.

Sleep has been historically viewed as a passive behavioral state until the identification of paradoxical sleep by Jouvet et al. [189], also known as REM sleep. With the development of EEG techniques in humans, sleep was subsequently described using standardized terminology and criteria developed by Rechtschaffen and Kales [190], recently updated and simplified by the American Academy of Sleep Medicine [191]. Sleep is generally assessed by polysomnography (PSG), which comprises EEG to measure frontal, central, and occipital cortical activity, electromyography (EMG) to measure muscle activity, and electrooculography (EOG) to measure eye movements. PSG recording is used to classify sleep into two main categories: REM sleep and non-REM (NREM) sleep. NREM sleep can be subsequently subdivided into 3 stages (i.e., stage N1 to N3) marked by progressive slowing and synchronization of electrical cortical activity, as observed on the EEG channels [191]. In healthy individuals, NREM and REM sleep alternate in cycles of approximately 90–110 min and are measured from the end of a REM sleep episode to the end of the subsequent one. The respective proportion of NREM and REM sleep during a sleep period differs between cycles. More N3 sleep (also known as slow-wave sleep) is typically observed in the early night and decreases with time spent asleep. On the other hand, the length of REM sleep bouts during each sleep cycle increases with the amount of time spent asleep. More precisely, the greatest amount of REM sleep is observed in the early morning hours.

The Two-Process Model

Initially proposed by Borbely [192], the two-process model of sleep regulation provides a theoretical framework describing the temporal organization and timing of sleep (Fig. 6.5). The homeostatic process, or Process S, reflects the increased sleep pressure as a function of the amount of time spent awake and its decline as a function of time asleep, similar to an hourglass effect. The circadian process, or Process C, regulates sleep as a function of time of day and depends on the action and timing of the SCN. As a result of the balance between the circadian and homeostatic processes, only a narrow set of sleep-onset times permits a continuous sleep period of about 8 h (i.e., sleep onset ~6 h before CBT minimum) [193–195]. Since its introduction in 1982, other components, such as sleep inertia and a NREM/REM sleep oscillator, have been added to the original two-process model, but the simple conceptual model developed by Alexander Borbely remains the most influential. It has been recently extended to predict alertness and vigilance around the clock [196].

The Homeostatic Process

For a long time, the sleep-wake cycle was considered to be regulated by the accumulation and elimination of a sleep-promoting substance in the organism. To date, many researchers have confirmed the existence of a homeostatic process regulating sleep, but have yet failed to identify a single sleep-related factor. The homeostatic process regulates sleep propensity as a function of the sleep-wake cycle length, independently of the time of day. Specifically, the homeostatic sleep drive or Process S increases when sleep is absent or restricted and conversely declines in response to sleep. Within a sleep period, the EEG slow-wave activity (SWA, 0.5–4.5 Hz) power measured during NREM sleep has been used to quantify Process S. It has been shown to be correlated with the duration of the prior wake period and to decline across the sleep period [197]. In contrast to Process S, the Process C only has a small influence on SWA [195]. The influence of waking on Process S has been estimated by quantifying the level of SWA during naps after various durations of waking [198]. Process S has been modeled by a saturating exponential function throughout the wake period, followed by an exponential decline during sleep [196]. Of note, sleep pressure dissipation during the sleep period is faster than sleep pressure accumulation during the wake period. Furthermore, the decline in Process S following sleep initiation is steeper in the first hours of sleep compared to the last ones. Based on the exponential decline of SWA throughout the sleep period, it has been estimated that after ~4.3 h of sleep, 75 % of the SWA needs would be fulfilled [195].

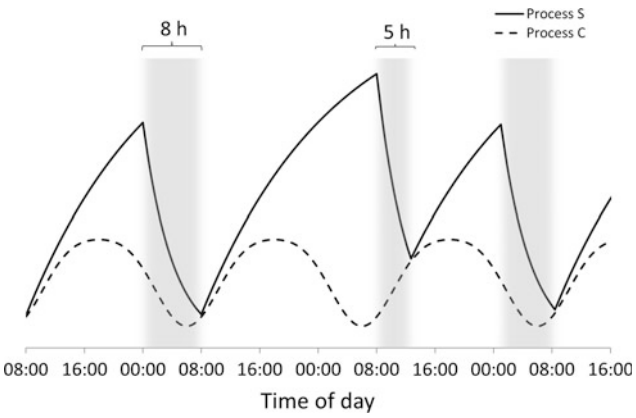

Fig. 6.5 The two-process model of sleep regulation. This model illustrates the interaction between the homeostatic (S) and circadian (C) processes in the control of sleep and waking [288]. Here, Process C represents the circadian variation in the strength of the wake signal, whereas Process S represents the dynamic changes in sleep pressure. During a standard day (e.g., 1st cycle; wakefulness: 08:00–00:00, sleep: 00:00–08:00), Process S (*continuous line*) increases exponentially during the wake period. During that time, Process C sends a wake signal that reaches its peak at the end of the evening. This allows the subject to remain awake for 16 consecutive hours. Sleep (*shaded bars*) is then initiated a few hours after the evening circadian peak of wakefulness. This time also corresponds to the start of the declining limb of the CBT cycle. Process S is at its peak at bedtime and then decreases exponentially throughout the night. At the end of the night, the circadian drive for waking is at its nadir, whereas the circadian drive for sleeping is at its peak. This allows the subject to remain asleep for 8 consecutive hours. When wakefulness is sustained during 24 h and sleep is initiated in the morning (as it is often the case for the first shift of a night shift worker), individuals usually wake up after a reduced amount of sleep. In this case, the present model predicts that about 5 h of consecutive sleep would occur (modified from Daan et al. [288])

Theta power (5–7 Hz) can also be used as an estimate of Process S and parallels sleep propensity during quiet wakefulness [199, 200]. As for SWA, the time course of theta power with time spent awake follows a saturating exponential function [201]. The rise rate of theta power measured during 40 h of sleep deprivation in humans and during enforced wakefulness in rats has been correlated with the SWA power increase observed in the beginning of a recovery sleep period [199, 200].

In the last decade, the synaptic homeostasis hypothesis has been proposed, in which Process S corresponds to the varying synaptic strength or neuroplasticity observed during the sleep-wake cycle [202]. More specifically, this hypothesis states that wakefulness is associated with synaptic potentiation and thus increased energy requirements in several cortical circuits caused by the acquisition of new information during waking. During sleep, the decrease in SWA observed during sleep is presumed to be caused by the progressive downscaling in synaptic strength during the sleep period. In this model, each synapse of a given network would undergo the downscaling process, but the relative weight of each synapse would be conserved, thus resulting in a decrease of energy and space requirement leading to a subsequent increased benefit for memory and learning capacity. Consistent with this hypothesis, it is now admitted that topographical differences exist between EEG derivations when measuring SWA and theta power during sleep and wakefulness, respectively. Indeed, frontal compared to occipital EEG derivations show increased SWA and theta power, especially in response to sleep recovery [203–205]. In addition, the regulation of SWA can be local, restricted to the specific learning cortex regions [203, 204, 206–209]. Brief local sleep can even be identified in local cortical region when rats are submitted to prolonged wakefulness even though the animal remains awake [210].

Some biochemical markers have been found to correlate with Process S and could represent chemical mediators of the homeostatic sleep drive. Such mediators are expected to accumulate during wakefulness and decline with sleep. Among others, adenosine [211], prostaglandin [212], interleukin-1β (IL-1β), tumor necrosis factor alpha (TNF-α), brain-derived neurotropic factor (BDNF), and epidermal growth factor (EGF) [213] have been described, but to date

adenosine represents the most studied candidate in the possible mediation of the homeostatic sleep drive [214]. First, extracellular adenosine accumulation is site specific. With time spent awake, adenosine starts to accumulate in the basal forebrain [211], followed by an increase in the frontal cortex [215]. Then, during sleep recovery, these levels decrease in the cortex and in the basal forebrain. Second, it was shown that sleep is induced by injections of adenosine or adenosine type 1 (A1) receptor agonists in the basal forebrain of cats [211] and by adenosine type 2a (A2a) receptor agonist injections near the VLPO area in rats [216]. This could be due to the fact that adenosine inhibits the release of acetylcholine (ACh) by cortical [217] and basal forebrain [218] neurons via a presynaptic mechanism. Third, caffeine is known to be an adenosine antagonist [219] inducing a reduction of sleepiness and EEG theta activity during wakefulness and a decrease of SWA during subsequent sleep [220]. Finally, in addition to the direct modulation of the frontal cortex activity, inhibition of the basal forebrain by the accumulation of adenosine can in turn promote sleepiness via its projection throughout the cortex. As described in the next section of this chapter, the basal forebrain is indeed part of the ascending arousal pathway regulating sleep.

The Circadian Process

One of the first indications that sleep and sleep propensity are regulated by the circadian system aroused from the work of Nathaniel Kleitman who noticed that staying awake for more than 24 h is usually associated with higher levels of alertness, the next morning in comparison with the levels observed at night [221, 222]. Following that simple but important observation, the circadian rhythmicity of sleep has been extensively studied by placing humans and animals in time-free conditions for several days [89, 223]. As we previously mentioned, without time cues, the circadian endogenous system free-runs with a period close to, but not exactly, 24 h [75, 224, 225]. Under these conditions, sleep propensity is modulated by the slowly shifting (i.e., free-running) rhythm of CBT [223].

The relative contribution of Processes C and S can be better appreciated when participants are studied under a forced desynchrony protocol described earlier in this chapter. Using this experimental paradigm, the main sleep period successively occurs at different circadian phases. Both Processes S and C can thus be mathematically separated and their specific contributions to the various sleep parameters can be observed. With such an approach, it was possible to determine that sleep latency (SL), total sleep time (TST), sleep efficiency (SE), and wake after sleep onset (WASO) have a strong circadian rhythm with increased sleep propensity around the time of CBT minimum [181, 193–195, 226, 227]. In addition, these measurements are influenced by the homeostatic process since sleep consolidation decreases with time spent asleep [194, 195]. The amount of REM sleep and REM sleep latency also follow a strong circadian rhythm with respective maximum and minimum in the morning, approximately 1–2 h following the bathyphase of CBT [181, 194, 195, 226, 227]. Process S can also influence the proportion of REM sleep which increases with time spent asleep. NREM sleep is controlled by the circadian and homeostatic system [195, 226]. The overall proportion of NREM sleep follows a circadian rhythm different from that of REM sleep, with the maximum values observed in the evening and early night [181, 195, 227]. With deeper sleep (i.e., N3 vs N1), there is a shift from an equivalent contribution of Processes S and C in N1, to an almost exclusive contribution of Process S in stage N3 [181, 194, 195, 226].

Subsequently, the introduction of the forced desynchrony protocol confirmed the existence of the circadian regulation of sleep in humans, independently of the homeostatic process. When scheduled to live on a 28- or 20-h rest-activity cycle, most sleep parameters present a circadian rhythm independent of their homeostatic regulation. Thus, this paradigm confirmed that a consolidated 8 h of sleep can only be obtained when sleep is scheduled approximately 6 h before CBT minimum, even though the time spent awake before each sleep period remains about the same [193–195].

The structure responsible for the circadian regulation of sleep was first evidenced following SCN lesions in rodents. In these nocturnal animals, the total duration of sleep over 24 h is similar to wild-type animals, but sleep is randomly distributed in small bouts throughout the 24-h day rather than consolidated bouts mainly during the day [228–231]. SCN-lesioned animals no longer present consolidated rhythms of NREM and REM sleep and display frequent sleep stages transitions and arousals [229, 231, 232]. The sleep recovery process (i.e., increased sleep duration and SWA) is not affected in SCN-lesioned [230] or arrhythmic [233] animals and confirms that Processes C and S arise from different neural substrates.

Although they are independent processes, evidence obtained from the manipulation of the sleep-wake cycle suggests that a higher homeostatic drive can reduce the circadian regulation of sleep. The circadian amplitude of sleep propensity was shown to be reduced in response to chronic sleep deprivation (i.e., 4-h vs. 8-h sleep opportunity per 24 h) in humans [234]. During 6 h of sleep deprivation, the SCN firing activity was reduced to 87 % compared to the baseline levels in rats [235]. During the following 7 h of

recovery sleep after sleep deprivation, the SCN neuronal firing activity during NREM and REM sleep was significantly reduced by about 40 and 30 %, respectively, compared to baseline levels [235]. During NREM sleep, the increase in SWA paralleled the decrease in SCN firing rate, both during normal sleep [236] and during recovery sleep after sleep deprivation [235]. Taken together, these studies suggest that the relative weight of Process C can change with the sleep-wake history, or at least with acute sleep deprivation.

The literature suggests that the SCN has both a sleep- and wake-promoting action depending on circadian phase. First, the majority of SCN-lesioned studies does not show changes in the total amount of sleep or sleep stages throughout 24 h [228–231], although some contradictory evidence has been reported [237, 238]. Furthermore, the proportion of sleep stages throughout the circadian day in SCN-lesioned animals is equivalent to the average 24-h rhythm observed in wild-type animals [228–230], although some evidence suggests that it might be different for REM sleep [231]. It is notable that, even when the homeostatic process is challenged, similar results can be obtained. For example, in the study of Mistlberger et al. [230], wild-type rats were sleep deprived for 24 h. Following this sleep deprivation, the authors analyzed the variation in the amount of sleep and sleep stages over 24 h. Surprisingly, they showed that during the first 12 h following sleep deprivation (corresponding to the habitual active period), the amount of sleep was lower than during the second 12 h (i.e., from 12 to 24 h after sleep deprivation, corresponding to the habitual rest period). In SCN-lesioned rats, when recovery sleep is compared to baseline levels, the proportion of sleep over wakefulness is increased to a constant level during the 24 h following sleep deprivation, with values in between the maximum and minimum levels observed in wild-type rats. This suggests that the SCN restrains sleep propensity during the habitual active period and promotes sleep during the habitual rest period.

Wake Maintenance Zones and Sleep Propensity Zones (The Interaction)

In entrained condition, humans sleep at night and are active during the day. However, when sleep timing was studied in time isolation environments, a bimodal distribution of sleep propensity was observed throughout the 24-h day, with two high sleep propensity and two high alertness zones [239, 240]. A first zone of high sleep propensity is observed at the end of the night, near the CBT minimum. A second peak in sleep propensity is observed in the early afternoon. Of note, in certain countries, naps are commonly taken after lunch and thus during this zone of high sleep propensity. Important, increased drowsiness, errors, and risk of accident have been reported to follow a bimodal patterns with increased levels late at night and in the afternoon [241–243]. Conversely, "wake maintenance zones" (WMZs) are also observed and are characterized by high levels of alertness and difficulty to fall asleep. The first WMZ occurs in the morning, approximately 3–4 h after waking up in the morning [239]. It is usually associated with increased productivity at work and thus represents a profitable timing to plan intellectually demanding tasks. A WMZ is also observed in the evening, approximately 1–2 h before the habitual bedtime. It was indeed shown that in a well-rested individual, advancing bedtime by 1 or 2 h could lead to longer sleep-onset latencies. This is especially important in sleep-onset insomnia patients who often try to advance their bedtime to lengthen their main sleep period, resulting in longer sleep-onset latencies and increased anxiety due to insomnia [244, 245]. These zones are important to consider in our daily schedule to more efficiently plan naps, activities, or when periods of sleep or wakefulness need to be acutely and temporarily displaced. Shifting the sleep-wake schedule, as observed in shift workers or after transmeridian travels, disrupt the normal temporal harmony between Processes C and S, a situation that can lead to reduced sleep duration and quality and lower levels of alertness.

In humans, the interaction between Processes S and C leads to the consolidation of an ~8-h sleep period at night and an ~16-h wake period during the day. In entrained conditions, sleep is initiated in the evening on the decreasing limb of the CBT rhythm, 1–2 h after the evening WMZ. The circadian regulation of distal heat loss, helped by the vasodilation effect of melatonin, is thought to facilitate sleep initiation at the usual bedtime [246]. The following exponential decline in Process S suggests that most of the deep-sleep needs are fulfilled within the first half of the night. Despite the reduced homeostatic need for sleep, the sleep period lasts approximately 8 h because Process C maximally promotes sleep in the second half of the night. Then, after awakening in the morning, sleep propensity is low as a result of the previous time spent asleep.

A phenomenon known as sleep inertia is responsible for the reduced alertness that can be observed in the time period following awakening [247]. Sleep inertia appears to depend on several factors such as sleep stages, time of day and amount of prior sleep deprivation, although its precise duration and severity requires further research [247, 248]. In the late morning, Process S is still low, sleep inertia has dissipated, and Process C starts to promote wakefulness, thus explaining the WMZ observed in the morning. In the afternoon, a zone of higher sleep propensity is observed as a

result of the time spent awake. In the evening, Process C keeps us awake until bedtime by promoting wakefulness maximally during the evening WMZ, even if Process S is elevated (Fig. 6.5).

Brain Regions Implicated in Sleep Regulation

During wakefulness, a series of ascending arousal pathways activate the thalamus and cortex. Two major arousal pathways have been defined. In the first one, cholinergic neurons from the pedunculopontine tegmental nucleus (PPT) and the laterodorsal tegmental nucleus (LDT) activate reticular and relay nuclei of the thalamus to facilitate thalamocortical transmissions. In the second pathway, monoaminergic neurons originating primarily from the locus coeruleus (LC), the ventral periaqueductal gray matter (vPAG), the DRN and MRN, the tuberomammillary nucleus (TMN), the lateral hypothalamus, and the basal forebrain contribute to diffusely activate the cerebral cortex. Sleep occurs when the arousal pathways are directly inhibited by the VLPO via GABAergic and galaninergic neurotransmitters [249]. The VLPO is innervated by inhibitory afferent fibers from nuclei of the arousal ascending pathways. This mutual inhibition between ascending arousal pathways and the sleep-promoting system (i.e., VLPO) can be viewed as a "flip-flop switch" that facilitates sharp transitions between wakefulness and sleep [58]. Orexin (ORX) neurons of the lateral hypothalamus area (LHA) reinforce the wake-promoting system by projecting to the cerebral cortex and to nuclei of both major arousal pathways. The VLPO also projects to ORX neurons and inhibits their action during sleep. Through its massive input to the DMH, the SCN modulates the activity of the VLPO, median preoptic nucleus, and ORX neurons [250]. Therefore, the central circadian clock directly acts as a time-keeping device for the sleep-wake cycle.

Transitions between NREM and REM sleep can be explained in a similar way by mutual inhibition of REM-on and REM-off centers [249, 251]. This switch is composed of the precoeruleus (PC) and sublaterodorsal nucleus (SLD, REM-on) mutually inhibiting the vPAG and lateral pontine tegmentum (LPT, REM-off). Other regulatory areas strengthen this mutual inhibition to facilitate NREM/REM transitions but are not necessarily inhibited by the opposite side of the NREM/REM switch. During REM sleep compared to NREM sleep activity, centers of the ACh arousal pathway including the LDT and PPT (REM-on) increase their activity, while the firing rate in most regions of the monoaminergic pathway completely ceases or decreases. These REM-off regions include the LC, DR/MR, vPAG, and ORX neurons. During both NREM and REM sleep, the VLPO remains active and maintains its inhibition over the monoaminergic arousal pathway.

Intriguingly, sleep centers can also influence the activity of the SCN. Compared to wake and REM sleep, NREM sleep was shown to reduce SCN neuronal activity in rats [236]. Specifically, SCN neuronal firing rate was negatively correlated with the amount of SWA (1–4 Hz) and sigma activity (11–14 Hz), both indicators of sleep homeostasis. This inhibition of SCN activity by SWA was confirmed by selective SWA and REM sleep deprivation experiments. The SCN receives afferent cholinergic projections from the PPT and LDT, serotonergic projections from the DR, and, to a lesser extent, noradrenergic projections from the LC, all implicated in arousal ascending pathways and in the generation of NREM/REM sleep cycling [236, 249, 252]. The presence of afferent neuronal connections to the SCN suggests that the sleep-wake cycle per se could modulate the circadian pacemaker [151].

Chronotype

Although humans are diurnal, individual preferences in the sleep-wake cycle and daytime activity timing are reported and are referred to as chronotype, or morningness-eveningness. Questionnaires have been developed to assess these differences and aim to identify the times of day at which the individual feels better to perform more demanding tasks and to sleep [253, 254]. The chronotype was reported to vary with age [255–257] and gender [258]. Teenager between the ages of 11–18 years tends to progressively delay their bedtime by ~2.5 h [255], a phenomenon that parallels pubertal development [256]. Of note, a progressive change from early to late chronotype has been observed starting at childhood, accelerating during puberty and peaking around the age of 20, with women (~19.5 years) reaching a maximum "eveningness" approximately 1.5 years before men (~21 years) and slowly declining thereafter. Nevertheless, from the age of 20–50 years, men generally present a later chronotype than women, with this sex difference disappearing after the age of 50 [257]. These changes during puberty are associated with a >60 % decrease in SWA and theta power from 9 to 18 years of age [259]. Thus, the homeostatic process is highly affected during adolescence. At that age, adolescents also tend to go to bed and wake up later, reflecting changes in chronotype throughout adolescence [260]. Moreover, later bedtimes could also enhance exposure to light at night and reduce light exposure in the morning and thus entrain the circadian system to a later phase.

Earlier circadian phases of melatonin and CBT have been reported in morning compared to evening chronotypes [261–265]. This difference was also recently reported in peripheral genetic markers, namely in the circadian oscillation of *Per1*, *Per2*, and *Rev-erbα* expression in samples of oral mucosa

[266]. Thus, it suggests that circadian entrainment differs between morning and evening types and influences the timing of their sleep-wake cycle. Of note, a variable-number tandem repeat in the human clock gene *Per3* has been correlated with chronotype, as well as with circadian rhythm sleep disorders [267–269], though not all studies report significant results [270, 271]. The shorter allele of *Per3* ($Per3^{4/4}$ vs. $Per3^{5/5}$) was associated with eveningness, but the acrophase of melatonin, cortisol, and *Per3* mRNA peripheral expression did not differ between carriers of the shorter and longer alleles [272]. Nevertheless, markers of sleep homeostasis were considerably different between *Per3* genotypes: The $Per3^{5/5}$ group was more affected by sleep deprivation, presented more theta and alpha EEG activity during wakefulness, and showed elevated SWA during NREM sleep compared to the $Per3^{4/4}$ group. These results suggest that the homeostatic drive for sleep also influences the timing of the sleep-wake cycle, which underlines the interaction between Processes C and S.

Most of the population is classified as neither morning nor evening type, but a significant proportion can be classified as moderate-to-severe morning or evening type [257, 273]. Patients suffering from delayed or advanced sleep-wake phase disorders can be considered as extreme morning or evening types, although chronotype questionnaires are not sufficient to make a diagnostic [274]. Delayed sleep-wake phase disorder has been associated with circadian gene polymorphism, including *PER3*, *CLOCK*, and *AA-NAT*, a limiting enzyme in melatonin synthesis. An association was also found between the c.3111T/C allele of the *CLOCK* gene and a latter bedtime as well as daytime sleepiness [275]. As for the advanced sleep-wake phase disorder, it has been associated with mutations in the *PER2* and *CK1*δ genes [276, 277] and which highlights the contribution of the circadian system to extreme chronotypes.

Conclusion

Most of us are unaware of the impact of the circadian system in our daily life until our sleep-wake cycle is displaced out of synchrony with this system. Unlike other species, humans can voluntarily and abruptly change their sleep-wake behavior or sustain wakefulness throughout the night, mainly because of social and professional incentives. Abrupt circadian misalignment, as observed in shift workers or in transmeridional travelers, leads to important acute performance and alertness impairments, as well as sleep disturbances [278–281]. Moreover, only a slight delay or advance of the endogenous circadian system relative to the scheduled sleep periods is sufficient to result in clinically relevant sleep-onset and sleep maintenance difficulties. This can lead to chronic sleep disturbances as it has been reported in patients suffering from insomnia, delayed sleep-wake phase disorder, advanced sleep-wake phase disorder, or non-24-h sleep-wake disorder [245]. Long-term exposure to circadian misalignment has been associated with increased risk of several medical conditions, including obesity, metabolic syndrome, diabetes, hypertension, gastrointestinal disorders, endometriosis, adverse pregnancy outcomes, cardiovascular disease, and cancer [282–285]. There is a high prevalence of comorbidity between psychiatric conditions and sleep-wake and circadian rhythm disorders. Interconnections between the circadian, sleep, and mood systems are such that deregulation of one system can lead to deregulation of the others [286]. Different pharmaceutical and non-pharmaceutical interventions have been proposed to treat circadian rhythm disorders including chronotherapy, light therapy, and melatonin and are usually planned based on the phase of the circadian system [245]. Further research is needed to increase the efficiency of these therapies and to develop a better way to assess human circadian rhythms in clinical populations.

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