The Circadian System: A Regulatory Feedback Network of Periphery and Brain

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Circadian rhythms are generated by the autonomous circadian clock, the suprachiasmatic nucleus (SCN), and clock genes that are present in all tissues. The SCN times these peripheral clocks, as well as behavioral and physiological processes. Recent studies show that frequent violations of conditions set by our biological clock, such as shift work, jet lag, sleep deprivation, or simply eating at the wrong time of the day, may have deleterious effects on health. This infringement, also known as circadian desynchronization, is associated with chronic diseases like diabetes, hypertension, cancer, and psychiatric disorders. In this review, we will evaluate evidence that these diseases stem from the need of the SCN for peripheral feedback to fine-tune its output and adjust physiological processes to the requirements of the moment. This feedback can vary from neuronal or hormonal signals from the liver to changes in blood pressure. Desynchronization renders the circadian network dysfunctional, resulting in a breakdown of many functions driven by the SCN, disrupting core clock rhythms in the periphery and disorganizing cellular processes that are normally driven by the synchrony between behavior and peripheral signals with neuronal and humoral output of the hypothalamus. Consequently, we propose that the loss of synchrony between the different elements of this circadian network as may occur during shiftwork and jet lag is the reason for the occurrence of health problems.

Almost all organisms are subject to cyclic environmental changes, enforcing a day-night rhythm on their physiology. Adapting to this cyclic world, organisms have evolved circadian systems synchronizing behavioral and physiological rhythms for optimal anticipation of changes in activity and food availability (15). In mammals, the circadian system consists of a central pacemaker, the suprachiasmatic nucleus (SCN), and of peripheral oscillators found in almost all cell types in brain and body that resonate with circadian cues originating from the SCN (61). The SCN is located above the optic chiasm (suprachiasmatic), through which it receives photic information. This photic information serves to synchronize the activity of the SCN to the daily light-dark cycle. The bilaterally paired SCN is composed of a dense network of ~20,000 interconnected neurons (115). A molecular clock mechanism inside each individual SCN neuron produces an ~24-h rhythm through autoregulatory transcription-translation feedback loops involving clock genes (80). Interestingly, for more than two decades it has been demonstrated that a molecular clock machinery of similar composition is also present in nearly all cells of the body. Importantly, these peripheral clock genes are mainly driven by SCN output, whereby, in principle, all SCN-driven outputs, hormonal (i.e., melatonin and corticosterone) (4), behavioral (i.e., activity and food intake) (21, 111), as well as autonomic and physiological (i.e., temperature and glucose), contribute to peripheral clock gene rhythmicity (11, 42). Since their discovery, clock genes have been shown to be involved in many different (cellular) functions in peripheral organs, from metabolic function to cell division (65).

Intercellular coupling seems to be critical for a robust, endogenously rhythmic SCN that distinguishes it from peripheral oscillators (70). SCN neuronal rhythmicity is translated into rhythmic release of SCN neurotransmitters, imposing a circadian rhythm onto target neurons. These target

neurons provide rhythmic behavioral, neuroendo-crine, and autonomic output, supporting a circadian organization of physiology. Through SCN endogenous activity, behavioral and physiological rhythms are maintained even in constant dark conditions (DD) (39). This allows the organism to anticipate day-night changes in the environment, best preparing the physiology for upcoming challenges. Since behavioral activity needs to coincide with, i.e., increased body temperature, higher circulating glucose levels, and elevated blood pressure, the main function of the SCN is to organize these physiological set-points, optimally adapting them to resting or active periods (48).

In this review, we will first discuss how the SCN is able to drive multiple rhythms in physiology and behavior and how the circadian system itself is synchronized by feedback from its own effectors. The perfect synchrony within this multiple oscillatory system raises the question of what happens when perturbing factors, such as changes in behavior elicited by shift work, disturb its equilibrium. Finding the answer has become more urgent, since desynchronous behavior has been associated with a wide range of pathology. The current hypothesis is that ill-timed lifestyle patterns, i.e., (high-fat) food intake, light pollution, shift work, or chronic jet lag, increase susceptibility to certain diseases. This is based on emerging data that this behavior, specific to humans, brings the individual in conflict with the endogenous rhythm of its biological clock. Here, we will discuss how such desynchronization importantly changes many physiological processes, from metabolism, immune function, and cardiovascular regulation to mental health. We will also address recent advances made in the treatment of disease specifically aimed at (re)synchronization of the circadian system.

Circadian Synchronization

The Clock

The SCN, as an autonomously rhythmic nucleus, distinguishes itself from other nuclei in the brain through its structure and function. It generates a rhythm in electrical activity, also in DD, with generally a higher frequency of neuronal firing during the (subjective) day compared with the night. The day-time peak in neuronal activity occurs equally in nocturnal as in diurnal animals, indicating that SCN activity alone does not determine behavioral activity. Neuronal depolarization during the day is driven by persistent Na^+ currents and oscillations in chloride pumps, K^+ channels, and Ca^{2+} pools. This elicits increased excitability of SCN neurons and facilitates spontaneous neuronal activity occurring even in the absence of synaptic drive, thus

giving the SCN its endogenous rhythm. At night, the reverse occurs, with neurons showing hyperpolarization, inhibiting neuronal firing and silencing the SCN (20).

This circadian rhythm in electrical activity regulates oscillatory transcription and translation of genes inside individual SCN neurons. These clock genes are part of an intrinsic oscillator, consisting of interlinked autoregulatory transcriptional-translational feedback loops. This molecular mechanism drives rhythmic, ~24-h expression patterns of core clock proteins necessary for the generation and regulation of circadian rhythms within individual cells (88). In mammals, protein complex CLOCK (circadian locomotor output cycles kaput)-BMAL1 (brain and muscle ARNT-like protein 1) bound to E-box promoters form the positive limb of the feedback loop. The negative limb consists of PER-CRY, heterodimers that translocate back to the nucleus suppressing their own transcription by inhibiting CLOCK-BMAL1 activity. Secondary loops are formed with the help of orphan nuclear receptors from the REV-ERB and ROR family, which fine tune the core clock machinery modulating the transcriptional feedback loop, thus contributing to the robustness of the molecular clock (for detailed description, see Ref. 80). This molecular circuitry is not only present in the SCN but in nearly all (peripheral) cells so expressing their own oscillations under influence of the SCN and peripheral signals (14).

Aside from this molecular machinery, the SCN also expresses numerous neurotransmitters involved in synchronizing and maintaining an endogenous circadian rhythm of the SCN, while also transmitting circadian timed signals to target neurons in the hypothalamus. Based on the anatomical location of these different neural populations, the SCN is generally divided into a ventrolateral and dorsomedial region. The ventrolateral SCN, associated with integrating external input, i.e., gastric-releasing peptide (GRP)- and vasoactive intestinal peptide (VIP)-expressing neurons receive direct retinal input via the retinohypothalamic tract (RHT). These neurons convey light-dark information to the rest of the SCN, with VIP critical in maintaining SCN synchrony (2, 38, 100). The dorsomedial SCN, where, i.e., arginine vasopressin (AVP) and prokineticin 2 (PK2) is expressed, is associated with generating robust circadian rhythms (1, 119). Also, GABA (co-expressed in GRP, VIP, and AVP neurons) is essential in synchronizing SCN neurons, adapting their activity through both excitatory and inhibitory modulation (20). Interestingly, exposure to long-day photoperiods changes GABAergic activity from inhibitory to excitatory, destabilizing SCN rhythmicity and possibly affecting its sensitivity to photoperiodic entrainment

(25). Also, neuromedin S, expressed in the majority of VIP and AVP neurons, is important for SCN synchrony (56). The ventral and dorsal SCN show elaborate neuronal interconnectivity (89), underlining the essential role of internal SCN communication.

Finally, coordination of rhythmicity among different cells within the SCN is achieved through intercellular coupling by, i.e., presence of gap junctions, glial network encoding, phase-dependent coupling through non-redundant VIP and GABA signaling, paracrine signaling, and through glutamatergic communication between the left and right SCN (for review, see Ref. 20).

The SCN Drives Rhythms in Behavior, Hormone Secretion, and Organ Function Through Its Neuronal Activity

Many SCN neurons have an endogenous rhythm in electrical activity (2, 20), which is the basis for the rhythmic release of SCN neurotransmitters at their terminals (14). However, in the rat, many neurons show a nonrhythmic-low constant neuronal activity. For example, via constant release of glutamate in the PVN, the SCN promotes melatonin release from the pineal, which can only be prevented by the diurnal rhythm of SCN-induced release of GABA in the PVN (83). Nonetheless, the majority of SCN neurons are more active during the light period than during the dark period (66). Light is a very powerful stimulus for neuronal activation of the retino-recipient portion of the SCN, both during the day as well as during the night (67). This activation results in inhibition of locomotor behavior, at least partially, by the release of Prokineticin 2 from its terminals (19). The daily light/dark cycle synchronizes SCN neurons to a precise 24-h rhythm, translated into appropriate behavior according to the time of day and the corresponding hormonal and autonomic signaling (14). This SCN output serves to drive the functionality of the organs both through the induced rhythm of clock and other genes in the organs (77, 81) and by the circadian rhythm of autonomic output (47).

The Hypothalamus as Integrative Neuronal Network Regulating Physiology

SCN projections reach many different target neurons-interneurons, endocrine neurons, preautonomic neurons, and neurons that gate physiologically relevant sensory information (13)–through which a wide range of effector organs are reached that have a somatotopic representation in the SCN (53). This not only provides an anatomical framework for the SCN to spread circadian signals to hypothalamic targets, it also allows the SCN to modulate the access of information entering the hypothalamus. Until recently, it was assumed that

the SCN would execute its functions by means of timed output that was only synchronized by the light/dark cycle. However, light is not the sole input or synchronizer of the SCN; melatonin (102), food (69), blood pressure (12), and locomotor activity (96) also have a direct effect on SCN neuronal activity or its phase. Somatic information is received through various direct projections from, i.e., the NTS, IGL, ARC, limbic system, and raphe nucleus (12, 62, 92). As we will elaborate in this review, we suggest the SCN is not a mere pacemaker but part of a large network of oscillators all functioning within series of feedback loops maintaining the organism in synchrony with its environment (FIGURE 1). In support of this notion, recent evidence of functional input to the SCN from circumventricular organs, brain stem viscerosensory nuclei, and hypothalamic integration nuclei (8, 12, 78, 92, 121) suggests that the SCN is influenced by peripheral signals entering the hypothalamus dependent on the physiological state of the organism, forming a complex integrative hypothalamic network regulating homeostasis (FIGURE 1).

Peripheral Oscillators Synchronized by the SCN

In the brain, apart from the SCN, autonomous cellular rhythms are found in the olfactory bulb and retina, whereas other structures, like the Arcuate nucleus (ARC), are able to express an independent rhythm for some time in vitro (30, 33, 108). The general view is that clock genes in non-brain tissues are not autonomously rhythmic; they derive their rhythm from the SCN or from SCN-driven processes. The loss of rhythm in peripheral organs following SCN lesions is probably due to the limited intercellular communication in peripheral organs and the loss of synchronizing corticosterone (4) or melatonin rhythm. This demonstrates the role of the SCN as synchronizer of peripheral rhythmicity, which is realized through various, still not fully understood pathways. First, autonomic output is capable of driving clock gene expression (107), although autonomic denervation of an organ does not abolish clock gene rhythmicity (17). Second, glucocorticoids influence clock gene expression, but adrenalectomy does not abolish rhythmicity (4). Third, food intake during the resting phase, although also affecting temperature and glucocorticoid rhythms, completely reverses clock gene expression in the liver, kidney, heart, and pancreas (21), showing that food intake is an essential synchronizing signal for peripheral organs. However, under fasting conditions, the rhythm in the liver persists for at least one cycle as it does in food synchronized SCN-lesioned animals (91). Fourth, SCN-lesioned animals sharing their blood circulation with intact animals develop a rhythm in

clock gene expression in the liver and kidney, indicating that circulating factors are important for their rhythmicity (35). Last, temperature, too, is capable of altering clock gene expression in the liver (11). Essentially, peripheral clock genes are guided by direct and indirect signals from the SCN and can be altered significantly in their expression and phase by behavior that is not in line with SCN signaling. This is adeptly illustrated by a recent study demonstrating that animals receiving food six times a day lose their rhythm in white adipose tissue in seven of nine tested oscillatory metabolic/adipokine genes but not the rhythm of clock genes. Abolishing the daily corticosterone peak also rendered the clock genes arrhythmic (105). This shows that metabolic genes do not only depend on clock genes for their rhythm but may depend on other processes as well. Nonetheless, supporting a fundamental role for clock genes in peripheral organ function are recent studies demonstrating that tissue-specific deletion of a single core clock gene fundamentally changes the functioning of the liver, white adipose tissue, or blood vessels (54, 81, 82). Seemingly, the rhythmicity or mere presence of clock genes is essential for the expression or suppression of regulatory genes present in tissues and organs, organizing a cascade of rhythms (reviewed in Ref. 52). The physiological importance of this molecular organization, synchronized to the SCN, can be concluded from observations showing reversed feeding rhythms inducing, along with inverted peripheral clock gene expression, steatosis in the liver (95). In the next paragraphs, we will discuss the importance of a circadian physiological equilibrium for the well-being of an individual and how changes in this balance may have deleterious effects on health.

From Circadian Synchronization and Balance to Divergence and Disease

Metabolic Information: Multiple Sources and Multiple Integration Sites

Such is the relevance of an optimal metabolic state, that almost all physiological systems react to metabolic cues. Since the availability of food supply is evolutionarily closely linked to the activity period, the biological clock–in interaction with the hypothalamus–plays an essential role in timing adequate circadian metabolic control. This only recently has become clear by experiments showing that the SCN receives strong feedback of peripheral metabolic signals. For example, the liver is capable of sending a starvation signal by fibroblast growth factor 21 (FGF21) secretion into the circulation, directly reaching SCN receptors. This induces a decrease of systemic insulin, an increase of corti-

costerone levels, an inhibition of growth, and a change in locomotor activity and reproduction (8).

An important relay in transmitting metabolic feedback is the Arcuate nucleus (ARC), the main metabolic integration center of the hypothalamus. For example, leptin, secreted by adipose tissue, may target the SCN via the ARC since ablation of leptin receptor-expressing neurons in the ARC leads to the disruption of the circadian rhythm in food intake (58). Similarly, deletion of ROCK1, a key kinase in the signaling of leptin, leads to severe diminishment of spontaneous daily locomotor ac-
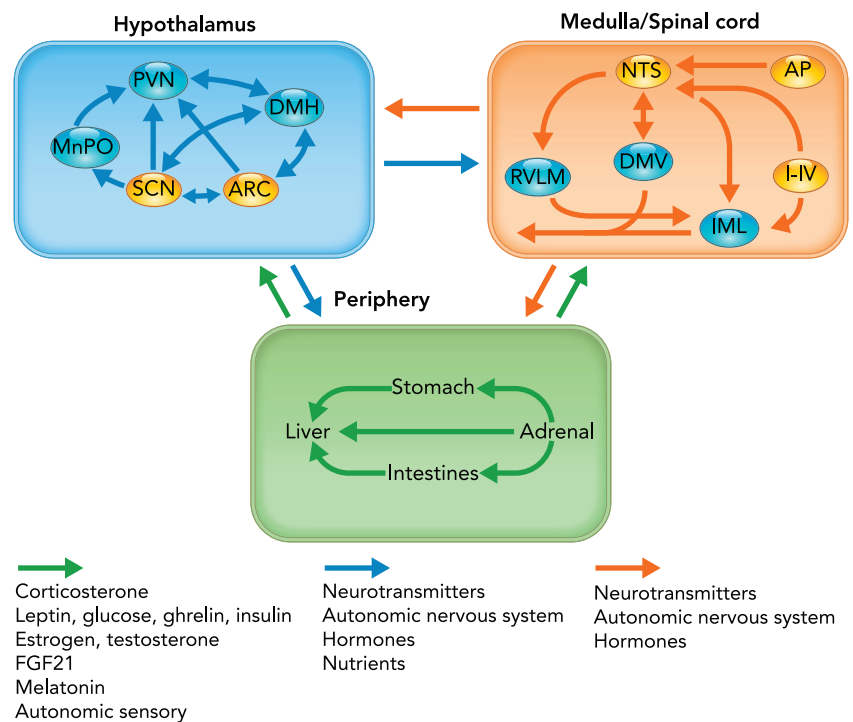

FIGURE 1. **Feedback networks of the circadian system**
Our hypothesis on the functioning of the circadian system consists of multiple interconnected feedback loops regulating physiology. Illustrated are three interconnected feedback loops: hypothalamus, brain stem/spinal cord, and periphery, within which, of course, are many other feedback loops on cellular, tissue, and organ level. *1*) Within the hypothalamus, the suprachiasmatic nucleus (SCN) sends timing signals to several target areas including the medial preoptic area (MnPO) for temperature regulation and reproduction; paraventricular nucleus (PVN) for hormone release and autonomic output; dorsomedial nucleus of the hypothalamus (DMH) as hypothalamic integration center; arcuate nucleus (ARC) as center for sensory metabolic information. All these nuclei are interconnected and the SCN receives direct feedback from all but the PVN. *2*) The brain stem/spinal cord feedback loop receives direct and indirect temporal information through the rostral ventral lateral medulla (RVLM), nucleus tractus solitarius (NTS), area postrema (AP), and the sensory layers lamina I–IV (I–IV) of the spinal cord. These nuclei function as integration centers for peripheral and central signals and are responsible for autonomic physiological reflexes transmitted to the dorsal motor nucleus of the vagus (DMV) and intermediolateral column (IML) that serve as autonomic output nuclei. *3*) The periphery receives temporal signals from the hypothalamus via autonomic output of the parasympathetic motor neurons in the DMV and via sympathetic motoneurons in the IML. In addition, circadian signals are also transmitted via hormones such as melatonin and corticosterone or by nutrients like glucose. The red ovals represent structures that receive autonomic sensory feedback such as the area postrema (AP), NTS, and the sensory layers lamina I–IV (I–IV) of the spinal cord; or hormonal and metabolic feedback from the circulation such as the AP, ARC, and SCN. Moreover, peripheral organs may communicate with each other via a circuit consisting of autonomic sensory signaling to the AP, NTS, and I–IV of the spinal cord followed by reflex automatic adjustment of autonomic output. Any disturbance or desynchrony between and within these circuits could, in time, potentially lead to pathology and disease.

tivity, suggesting an essential role for metabolic feedback to the ARC in maintaining circadian rhythmicity (44, 58). Moreover, circadian control of temperature is dependent on concurring AVP and α-MSH signaling from SCN and ARC to the medial preoptic area (MnPO), orchestrating a time-dependent temperature decrease (37). This illustrates the need for the SCN to synchronize with metabolic cues for adequate control of physiology. These metabolic cues are also potentially important for rhythmicity, as illustrated by observations that metabolic signals originating from the ARC (121), the lateral hypothalamus (LH) (5), or the intergeniculate leaflet (IGL) are capable of changing the activity of the ventral SCN (92). Considering the ventrolateral region is associated with synchronizing the SCN, this could provide a pathway for the synchronizing effect of food on the SCN. This, for example, was shown in hypocaloric food-restricted animals, whereby, in contrast to normocalorie-fed animals, feeding cues were able to alter SCN clock gene oscillations (68).

In vivo lesioning studies have demonstrated the importance of the network properties of the mediobasal hypothalamus in maintaining circadian rhythmicity (27), thus confirming early studies showing loss of activity rhythm due to knife cuts posterior to the SCN (71). Altogether, this argues for a system where the SCN coupled to other hypothalamic nuclei (and peripheral organs) form a network of oscillators essential for maintaining circadian rhythmicity.

This may explain why long-term desynchronous metabolic feedback has a deleterious effect on the circadian system and on health. In rodents, high-fat diet or food intake during the rest phase has been shown to desynchronize and dampen clock gene rhythmicity (21), leading to obesity, insulin resistance (23), and cardiovascular disease (81), thus providing a link as to why these diseases, including cancer, have a high incidence in shift-workers (22, 51). Another example is humans with night-eating syndrome, where high caloric intake during the resting phase disrupts the normal circadian pattern and results in a tendency to develop obesity (43). (For a detailed review of metabolic desynchronization and consequential health effects, see Ref. 26.)

Since behavior and SCN clock genes can also be synchronized to food (55, 68), food may have a protective effect on desynchrony. This is shown in a rat model of shift work, whereby restricting food intake to the normal activity period while working in the rest period induces a significantly lower weight gain and an increased insulin sensitivity compared with ad libitum shift-worker animals (94). This shows that the hypothalamic circadian system, with the SCN at its core, is a complex, reciprocally connected network that organizes metabolic homeostasis of the body and is capable of being (de)synchronized through peripheral signals (FIGURE 2). In the next paragraphs, we will give examples of how SCN-driven physiological rhythms are not driven in isolation but depend on each other to become fully rhythmic.

Temperature: Circadian and Metabolic Influences

Ahead of the active phase, core body temperature (Tb) starts to increase, independent of locomotor activity, whereas Tb drops just before activity cessation in the resting phase (86, 98). The central role of the SCN in the metabolic and temperature-regulating network becomes clear when it is noted that SCN lesions prevent not only temporal Tb rhythmicity but also fasting-induced Tb decrease (60). This temperature decrease is preceded by a drop in metabolic rate, hinting at a significant role for the ARC. Hereby, numerous hypothalamic nuclei, i.e., dorsomedial hypothalamus (DMH), ventromedial hypothalamus (VMH), ARC, and MnPO with the SCN at its core, are all jointly involved in temperature control (72), revealing a complex temperature-regulating network. On the basis of observed interactions between the SCN and ARC (36, 93), it was shown that Tb rhythm depends on interplay between temporal signals from the SCN and metabolic signals arising from the ARC (37). Not only is an SCN-driven rhythm of ARC neurons essential for this, it also requires a synchronized release of SCN vasopressin and ARC α-MSH neurotransmitters in the MnPO to organize diurnal temperature decreases in rats. Lesions of specific ARC neuronal populations or severance of reciprocal connections between the SCN and the ARC critically modify circadian patterns in corticosterone release, food intake, temperature, sleep, and locomotor activity (58, 117). These observations suggest that uncoupling the SCN from metabolic integration sites like the ARC may be an important factor for circadian desynchronization and the development of metabolic disease.

The Hypothalamic-Pituitary-Adrenal Axis and the Preparation for Activity and Food

The hypothalamic-pituitary-adrenal (HPA) axis is under strong control of the SCN. SCN-induced release of vasopressin in the beginning of the sleep phase has a strong inhibitory influence on the secretion of ACTH and corticosterone (49), whereas diminishing this inhibitory input toward the beginning of the active period induces the diurnal peak in corticosterone, preparing the animals for activity onset. Interestingly, crepuscular animals, active at dusk and dawn, have two

SCN-driven peaks of corticosterone (50). Closely associated with, but not driven by, corticosterone is the peak of circulating glucose. Corticosterone signaling to the ARC reduces hepatic insulin sensitivity (122), creating a perfect harmony between the corticosterone and glucose peaks, whose rhythms are synchronized by the SCN. This observation also explains why such strong metabolic alterations are observed in hypercortisolism. The same is seen in stress disorders or in chronic jet lag/shift work mimicking the effects of chronic stress, causing increased glucocorticoid production, which is correlated with developing diabetes and obesity (52).

Locomotor Activity Fine-Tunes SCN Rhythmicity

Locomotor activity, closely associated with arousal, has an effect on SCN neuronal activity and synchronization of the circadian system, although at a much lower intensity than light. Nevertheless, activity has been shown to directly inhibit the neu-
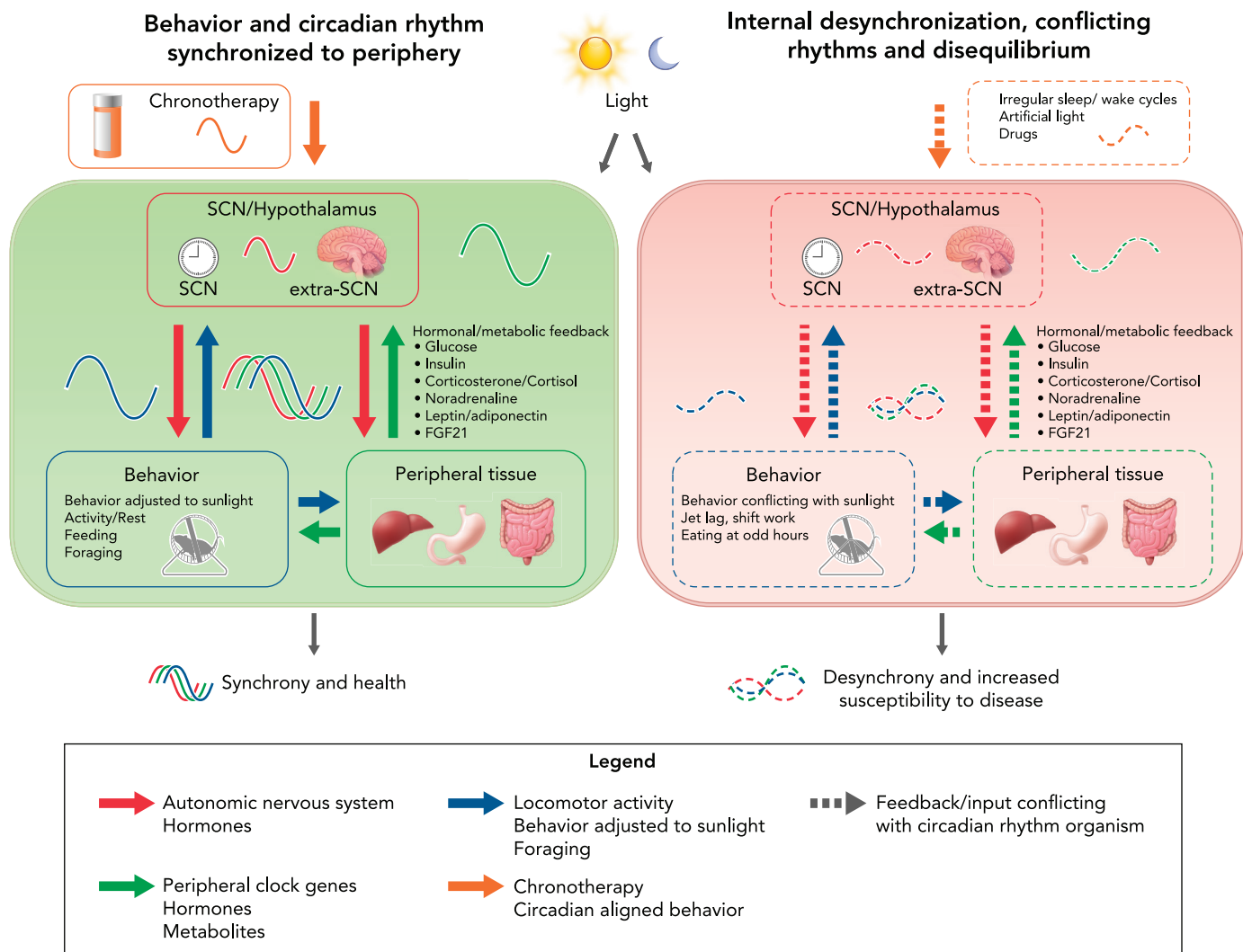

FIGURE 2. Proposed organization of synchrony in the circadian system
Whether an organism is in equilibrium depends on whether the multitude of circadian rhythms expressed are in synchrony or oppose each other. A synchronized and healthy situation is depicted on the *left* where light synchronizes the activity and rhythm of the SCN. The SCN transmits this rhythm via the autonomic nervous system (ANS), hormone secretion, and behavior to the body, thus synchronizing the periphery and adjusting the physiology according to time of day (red arrow). In turn, the periphery sends feedback to the brain via metabolites, hormones, and autonomic sensory pathways (green arrow). The periphery, through release of hormones and metabolites and in concert with autonomic signaling, also affects locomotor activity and foraging behavior (green arrow). Behavior, through locomotor activity or eating behavior, feeds back to the periphery and the brain (blue arrows), amplifying circadian rhythmicity and synchrony. When, as depicted on the *right*, the light-dark cycle, behavior, and peripheral signals do not align with that of the SCN or the hypothalamus (broken arrows), the deleterious feedback interferes with the circadian system equilibrium, which in the long term could potentially lead to desynchrony and development of disease. Chronotherapy in the form of circadian-timed drug administration or synchronizing sleeping/eating behavior with the light-dark cycle (orange arrow) can augment circadian system resynchronization, potentially reversing pathology and reducing disease.

ronal firing in the SCN, especially during the subjective day (96, 120). Thus activity may be a potential (de)synchronizer of the circadian system. The synchronizing property is seen in daily forced locomotor activity in DD: when the forced activity is halted, free-running rhythm runs closer to 24 h than the prior basal free-running rhythm, even in Vipr2$^{-/-}$ mice (45). Activity may also feed back on areas outside the SCN; for example, the Raphe nucleus is known to be important for the synchronization of SCN neuronal activity through its serotonin projections, i.e., by desensitizing the SCN to light (62, 109). The SCN and hypothalamus take turns to synchronize the Raphe nucleus through locomotor activity and corticosterone; they both target and induce rhythmicity in serotonin synthesis (62). This is significant because it illustrates how the SCN receives feedback related to its own output and is able to synchronize, through locomotor activity and corticosterone release, serotonin synthesis. Another example is melatonin secretion; driven by the SCN at night, it also enforces the night signal through melatonin receptors in the SCN (9, 87). These examples illustrate how important amplification of the proper circadian rhythms can be for maintaining or restoring adequate physiological function.

(Re)synchronizing the Cardiovascular System

Cardiovascular incidents follow a circadian rhythm that has its highest incidence early in the activity period (73), suggesting the involvement of the circadian system in cardiovascular pathology. Recent studies have also emphasized a role for peripheral clock genes in cellular processes associated with blood pressure control. Within the adrenal, absence of cry has been associated with hyperaldosteronism and hypertension (24). In the kidney, the absence of per1 was associated with the regulation of renal epithelial sodium channels (34). Other clock genes have been implicated in vascular endothelial function (18, 113) or in thrombogenesis (116), with potential relevance for humans (97). Still, the prevention of vascular pathology is dependent on the integrity and rhythmicity of the circadian system as a whole and not the mere consequence of bmal1 deficiency or clock mutation alone (28).

Chronic changes in the SCN have been observed in both hypertensive humans and rats (29, 84), showing a link between an altered circadian system and disease. The importance of the SCN not only as a master clock but also as an integration site in the physiological circuits regulating blood pressure is illustrated by the observation that the SCN receives cardiovascular feedback via the NTS (12). This indicates that untimely changes in blood pressure, which may occur in shift work, jet lag, or long activity at night, may disturb the functionality of the SCN via this feedback pathway.

In a mouse model of induced cardiac hypertrophy, desynchronization through shortened light-dark cycles significantly increased cardiac pathology compared with synchronized animals. Restoration of the natural circadian rhythm fully reversed the pathophysiology seen in those animals (64). This suggests that circadian desynchrony can contribute greatly to the progression of organ dysfunction and development of disease, whereas restoration of circadian rhythmicity potently reverses pathology. In recent years, chronopharmacology has thus developed into a potentially effective way of treating cardiovascular disease associated with circadian desynchronization; e.g., the treatment of "non-dipper" hypertensive patients is more effective when the therapeutic window of anti-hypertensive drugs is aimed to match the physiological trough in blood pressure (40). Also, evening administration of low-dose aspirin significantly reduces morning platelet reactivity and thus the risk of thrombo-embolic events, which peak early in the morning (7). Interestingly, repetitive nighttime melatonin administration, known to amplify the rhythm of melatonin secretion via an action on the SCN (9), substantially reduces blood pressure in hypertensive patients (99). It can be inferred that synchrony between the cardiovascular system and the SCN is essential for homeostasis, whereas desynchronization within this system could ultimately result in the development of cardiovascular disease.

Synchronizing the Immune System

The circadian system has a strong influence on the immune system, e.g., mortality is greater when bacterial endotoxin LPS (lipopolysaccharide) is given to rodents during the night, a time that coincides with increased pro-inflammatory cytokine production after LPS (63). It is suggested that the SCN is incorporated into a regulatory circuit between the immune system and the brain, as shown by the activation of the SCN following an inflammatory stimulus. Ablation of the SCN amplifies the innate immune response several fold, suggesting inhibitory influence of the SCN (32). Clock genes in immune cells also play an important role in the immune response (101), emphasizing the role of the circadian system. Cytokine interferon-α, used in cancer treatment, has a strong disruptive effect on locomotor activity and body temperature as well as on clock gene expression in the SCN. These adverse effects are for a large part prevented by changing the time of administration (76), emphasizing the strong interaction between circadian regulation and the immune

system. Circadian desynchronization induced by shift work in rats is associated with an enhanced inflammatory response that was prevented by synchronizing food with the normal feeding time (31). For that reason, therapies limiting food intake to the normal activity period may help to balance the immune response and may prevent development of inflammatory diseases.

Since the discovery of oscillatory clock gene expression in tumors, chrono-pharmacological cancer treatment, i.e., finding the optimal times for drug administration based on circadian variation in drug pharmacokinetics, efficacy, and tolerance, has received much attention and appears promising. Studies show that chrono-chemotherapy improved therapeutic outcome and survival for numerous types of cancer in humans (46).

Aligning the Reproductive System

Looking at the reproductive cycle, the SCN is essential for integrating and synchronizing all neuroendocrine signals involved in initiating a well timed GnRH-LH surge (103). Several studies have shown the importance of direct SCN signaling, through VIP (106, 110) and vasopressin (79), in interaction with the kisspeptinergic system (103), for accurately timing the LH surge. However, reproduction does not solely depend on a correctly functioning SCN: without peripheral signals, i.e., about the metabolic state of the body, a reproductive cycle cannot be completed. A liver-neuroendocrine signaling pathway has recently been described through which FGF21, a fasting-induced hepatokine, acts through the SCN, suppressing the vasopressin-kisspeptin signaling cascade and thereby inhibiting ovulation during starvation (78). Other fasting-elicited hormonal changes, such as low leptin levels, also prevent a successful cycle (6). These examples show that not only circadian timing but also synchronized metabolic and physiological feedback is essential for a successful reproductive cycle.

Circadian Dysfunction in Psychiatric Disorders and Their Treatment

People suffering from depression, bipolar disorder (75), anxiety, or schizophrenia (118) exhibit fatigue, changes in sleep, appetite, and body weight, and circadian desynchronization. Patients exhibit dampened temperature rhythms (3), altered cortisol levels (114) (itself a predictor for the course of illness), and melatonin secretion (57). Other visible features of chronic circadian desynchronization associated with psychiatric disorders are metabolic syndrome, obesity, diabetes, hypertension, and dyslipidemia (112), all contributing to premature death occurring up to 10 years earlier compared with the general population.

Clinically depressed individuals exhibit clock gene dysregulation in specific brain areas, abnormal phasing of clock gene expression, and potentially disrupted phase relationships between individual circadian genes, suggesting a desynchronization within the circadian network (59). This is supported by the observation that multiple simultaneous chronotherapeutic interventions aimed at synchronizing the circadian system, in the form of bright light therapy and advancing the sleep phase, are an effective treatment for sustained improvement in severely depressed patients (16). Interestingly, many pharmacological agents for the treatment of psychiatric disorders, i.e., olanzapine, quetiapine, have large cardiovascular and metabolic side effects but are under-acknowledged and undertreated (104). Synchronizing the circadian system by administration of nightly melatonin significantly decreases drug-induced proneness to obesity and blood pressure alterations (90). This finding reaffirms an important role of melatonin in synchronizing the circadian system and prevention of cardiovascular and metabolic pathology, also in relation to adverse effects associated with antipsychotic drugs.

Conclusions and Future Directions

Here, we have argued that, with the SCN at its center, the circadian system forms a coupled multi-oscillatory system, wherein each part receives a multitude of signals, fine-tuning circadian rhythmicity. The oscillatory organization of this system is maintained through the central rhythm of the SCN that, through hormonal, neuronal, or behavioral signals, fine tunes bodily functions to the activity or resting period. The autonomous rhythm of the SCN is augmented and fortified through cerebral and peripheral feedback, making the circadian system more robust and less prone to environmental variations. However, long-term perturbations through drug use, untimely light, or disorderly behavior will induce peripheral signaling capable of disrupting this harmony, rendering an individual more susceptible to desynchrony and disease. This makes clear that the SCN does not only function as a sophisticated timing mechanism but is integrated in multiple oscillatory feedback circuits involved in the regulation of physiological and behavioral functions.

The proneness of oscillatory networks to desynchronization has recently been analyzed through a mathematical model of the evolution of feedback networks in bacteria, fungi, and drosophila (74). The robustness of a network was demonstrated to be dependent on the number of interconnections and the number of regulators per connection, with an increasing number of interconnections and reg-

ulators associated with an increase in robustness. This is also illustrative for the circadian systems functioning, e.g., by the molecular feedback loops controlling clock gene rhythmicity inside individual SCN neurons. In turn, these weakly rhythmic individual neurons (41) function inside a larger coupled network, making up the SCN, driving a common rhythm and regulating its circadian output. Considering the here-reviewed studies, the SCN is in turn incorporated in a larger hypothalamic network of oscillators integrating peripheral signals. Finally, behavior and external stimuli like food intake or drug (ab)use also have their place in the feedback circuitry of the organism, adjusting adequate circadian function (FIGURE 2). The complex nature of the circadian system makes it robust and capable of withstanding brief erroneous feedback, but years of conflicting feedback, ill-timed behavior, or chronic jet lag/shift work will increase susceptibility to pathology and disease. The complex nature of this circadian network also suggests that it will take time before the full complexity of the circadian system will be understood. We and others (10) suggest that a holistic approach will be crucial in filling in the many gaps in knowledge of the circadian system. Many current developments in (molecular) chronobiology, such as in vitro analysis, conditional knockout animals, and optogenetics are doubtlessly invaluable and indispensable in present chronobiology research. However, considering the complexity of the circadian network, caution should be exercised in extrapolating conclusions from such investigations into in vivo models. For example, despite in vitro data suggesting the direct production of NAMPT via CLOCK/BMAL1 (85), it has been observed that, in animals eating during the light period, NAD^+ and NAMPT, together with certain metabolic genes, do not follow the inversion of rhythm in core clock genes like CLOCK/BMAL1 (95). These observations indicate that in vivo, alternative essential molecular relationships prevail, likely driven by other components of the circadian system, such as melatonin or corticosterone. Testing isolated brain areas in vitro or selectively activating small populations of neurons in vivo through optogenetics gives insight into an isolated stimulus response but fails to give a full picture as to how systemic physiological processes are truly regulated. Basic physiological experimentation and research is thus still very important for an understanding of physiological functions of the organism as a whole. ∎

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