The neurobiology of circadian rhythms
Eddy A. Van der Zeet, Gretha J. Boersma and Roelof A. Hut

*Departments of Molecular Neurobiology,
*Neuroendocrinology and *Chronobiology, Center of
Behavioral and Cognitive Neurosciences, University of
Groningen, Haren, The Netherlands

Correspondence to Eddy A. Van der Zee, Molecular
Neurobiology, Center of Behavioral and Cognitive
Neurosciences, University of Groningen, Kerkslaan 30,
9751 NN Haren, The Netherlands
Tel: +31 0 50 3632062; e-mail: e.a.vander.zee@rug.nl

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Purpose of review
There is growing awareness of the importance of circadian rhythmicity in various
research fields. Exciting developments are ongoing in the field of circadian neurobiology
linked to sleep, food intake, and memory. With the current knowledge of critical
‘clock genes’ (genes found to be involved in the generation of circadian rhythms) and
novel techniques for imaging cyclic events in brain and peripheral tissue, this field of
research is rapidly expanding. We reviewed only some of the highlights of the past
year, and placed these findings into a mutual circadian perspective.

Recent findings
Recent findings on the organization of the circadian clock systems are addressed,
ranging from the retina to the suprachiasmatic nucleus and peripheral organs. Novel
developments in sleep, food intake, and memory research linked to circadian aspects
are discussed.

Summary
The neurobiology of circadian rhythms is pivotal to the orchestration of the temporal
organization of an individual’s physiology and behavior. Endogenous circadian timing
systems underlie coupling and uncoupling mechanisms of many neuronal and
physiological processes, the latter possibly inducing health risks to the organism. The
integration of sleep, food intake and memory in a circadian setting has clear potential as
a systems neurobiology line of research.

Keywords
energy homeostasis, memory, sleep, suprachiasmatic nucleus

Introduction
Circadian rhythms allow the organism to anticipate and respond to environmental changes and adjust accord-
ingly. Circadian timekeeping systems in mammals are known to be organized in a hierarchical multioscillator
network with the suprachiasmatic nucleus (SCN) acting as the central pacemaker (Fig. 1). This brain region,
located in the ventral part of the hypothalamus, drives daily (circadian) rhythms. In several neurobiological
aspects the SCN is a remarkable brain region, with an unusual high level of intercellular communication. It can
be viewed as a programmable and flexible internal timekeeping system. Data on the expression of proteins novel
to the SCN appear regularly. For example, the SCN was shown to be one of the few adult brain regions with dense
doublecortin (DCX) expression [1], DCX plays a role in neuronal and synaptic plasticity, and DCX may mediate
rhythmic changes in SCN synaptic organization that underlie day/night changes in electrical signaling. Such
reports make clear that the neurochemistry of the master clock has not been fully mapped yet.

Nowadays, it has been shown that circadian oscillators exist in most regions of the brain, the retina, and many
peripheral tissues such as the liver [2] (Fig. 1). It also became apparent that most body tissues contain circadian
oscillation mechanisms that may uncouple from the SCN’s influence only under specific conditions. The food
entrainable oscillator (FEO), which becomes apparent under restricted feeding conditions, is illustrative for an
uncoupling process from the SCN. Restricted feeding conditions affect clock gene expression in many regions
of the hypothalamus [3], but not all known clock genes seem to be involved [4]. Although the opposite has been
suggested [5], it is now clear that the FEO does not depend on the canonical circadian molecular network
and that it cannot be localized to the dorso-medial hypothalamus (DMH) [6]. Possibly, the FEO does not
reside in a single brain region but in a neuronal network. This network could be the septo-hippocampal-thalamo-
hypothalamic circuit, which was found to be activated 3 h before food anticipation [7]. Nevertheless, the quest for
finding the site and mechanism of the FEO remains open.

Circadian rhythms and the retina
Zeitgebers (external stimuli phase shifting and entraining the intrinsic circadian rhythm) synchronize the circadian
rhythms to the environment, with light being critical for the SCN and food timing for the FEO. Within the SCN, neurons generate oscillations of a period of approximately 24 h that are synchronized (entrained) to the external light/dark cycle via light input from the retina. The observation of light entrained retinally-degenerated mice led to the discovery of the novel photopigment melanopsin [8,9], which was shown to be the prime photopigment driving circadian light entrainment and other nonimage forming light responses. Melanopsin is found in intrinsically photosensitive retinal ganglion cells and in a novel human cone type [10]. The bi-stability of melanopsin (similar to insect opsins) [11,12,13] opens the possibility of photosensitization as a tool to enhance circadian entrainment.

The circadian oscillator in the retina has not yet been located to a specific cell type, but it is essential for rhythms in retinal sensitivity [14,15]. This observation may in part explain the mechanism by which the circadian system modulates its own photic input [16,17].

### Circadian clocks and output pathways

Essential in the neurobiology of circadian rhythms is the wiring of the SCN. Gradually it is better known how the SCN communicates to other brain regions to impart or entrain circadian rhythmicity in behavioral and physiological processes (Fig. 2; [18] and references therein). The SCN transmits light information to peripheral organs such as the liver via autonomic innervation [19], which can lead to changes in liver clock gene expression unrelated to the expression of liver output genes [20]. The functional relevance of such communication between the SCN and peripheral organs is as yet unknown.

Recently, an interesting novel SCN output pathway to the ventral tegmental area (VTA) via the median preoptic nucleus (MPON) has been described [21]. This projection may function as the circadian regulator of behavioral processes such as arousal and motivation, further bridging well known behavioral observations on reward-related actions and circadian rhythmicity.

Peripheral tissues exhibit their own distinct pattern of phase distribution of clock and clock-controlled genes (e.g. [22]). Why are peripheral clocks needed in the presence of a central brain clock? One explanation is that these organs require independence from the SCN-derived rhythm to function optimally. An example is the
finding of the liver clock, driving a daily rhythm of hepatic glucose export that counterbalances daily food intake during sleep [7*]. Another example is the hippocampus, pivotal in neuronal plasticity, learning, and memory processes, which shows rhythmic gene expression relatively independent of the SCN (Fig. 2). This allows for the initiation of intrinsic rhythms necessary for time-of-day dependent memory formation, which can and probably needs to be desynchronized from the SCN rhythm.

**Circadian rhythms and sleep**

Perhaps one of the most conspicuous features of the circadian system is sleep–wake cycle regulation. Although the fundamental function of sleep is one of the most important open questions in neurobiology [23], several recent insights shed light on its regulatory pathways. The SCN is essential in sleep timing and the dorsomedial aspect of the SCN seems specific for the regulation of rapid eye movement sleep [24]. A direct influence of light on sleep architecture is mediated by melanopin containing retinal ganglion cells. Melanopsin knock-out mice (OPN4<sup>−/−</sup>) show disrupted sleep architecture [25–27] which might be explained by direct projections of melanopsin containing ganglion cells to the ventrolateral preoptic nucleus (VLPO) [28], a hypothalamic area involved in sleep–wake regulation via GABA-ergic projections to lateral hypothalamic orexin (hypocretin) neurons [29,30]. Surprisingly, sleep architecture is also affected by genetic make-up [31–33], and circadian clock genes were found to regulate both circadian and homeostatic components of sleep regulation [34,35]. Insights in molecular sleep regulation and circadian interactions arise from *Drosophila* studies [36–38]. Translation of these findings to mammalian sleep regulation might be a hazardous operation, partly because different definitions of sleep are used in both fields.

**Circadian rhythms, energy metabolism and food intake**

The exact role of the clock genes in food intake regulation is currently being elucidated. Per2<sup>−/−</sup> mice were found to lack the typical light/dark food intake pattern. Additionally, these Per2<sup>−/−</sup> mice do not develop food anticipatory behavior, whereas Per1<sup>−/−</sup> and wild type mice do [39]. Furthermore, Per2<sup>−/−</sup> mice lack the α-melanin stimulating hormone (α-MSH) pulse (a neuropeptide inhibiting food intake during the light phase), typically seen before the light phase fasting period [40]. Roles for circadian clocks in energy balance and in the pathological consequences of its disturbance have been suggested. Several studies show that sleep loss leads to increased metabolic syndrome risk (reviewed in [41]). Additionally, misalignment of circadian and behavioral cycles, as is seen in shift work, induces a risk for diabetes and cardiovascular disease [42*], and neuronal PAS domain protein 2 and Per2 clock gene mutations were also linked to metabolic syndrome [43]. Evidently, the circadian clock influences an individual’s metabolic well being; however, the reverse also seems true. Deregregation of energy balance, as in metabolic syndrome, altered the expression of several clock genes in brainstem and liver [44,45*]. It remains debatable whether circadian misalignment is cause or consequence of metabolic diseases.

Apart from the mentioned interactions between food intake and circadian rhythmicity, a common link between circadian oscillators and food intake, in the brain reward pathway. Circadian mechanisms are important for the development and expression of reward-related behavior. Withdrawal from chronic treatment results in desynchronization from the SCN rhythm in reward-related brain regions [46]. The suggested link between feeding and reward evokes contradicting views on whether hyper-responsiveness or hypo-responsiveness of the reward system leads to overeating (reviewed in [47]). This contradiction might be explained by a differential effect on anticipatory, circadian regulated food intake, and consummatory feeding behavior [48*]. The notion of a robust influence of circadian oscillators on food intake anticipation in combination with the correlation between food anticipatory activity and the reward system suggests an interaction between circadian rhythmicity, reward, and energy metabolism (Fig. 3). This interaction may be mediated by orexin, an orexigenic neuropeptide that stimulates food intake and wakefulness. In Orexin<sup>−/−</sup> mice cataplexy was powerfully triggered by anticipation of highly rewarding meals, whereas cataplexy was rarely triggered by standard meals, suggesting a role of the reward system in the feeding–wakefulness interaction [49*].

**Circadian rhythms, learning and memory**

The gap in our understanding of how the biological clock and circadian rhythms affect memory processes is being bridged. In general, the sleep–wake cycle supports acquisition (learning) during wakefulness, and promotes memory consolidation during sleep. In *Drosophila*, new findings demonstrate that synaptic proteins increase during wakefulness and decline during sleep [60*], which coincides with increasing and decreasing synapse numbers [51*]. These studies support the synaptic downscaling hypothesis as a function of sleep. Disruption of sleep–wake patterns and circadian organization of behavior are decremental to memory. For example, arrhythmic Siberian hamsters, delayed novel-object learning was impaired [52]. This suggests that hippocampal-dependent learning
The reciprocal crosstalk between the circadian timing system, sleep-wake regulation and metabolic regulation is crucial for an organism’s well being. It should be noted that memory, learning past, present, and future, on which many decisions and actions of an organism in maintaining energy homeostasis are based, is directly linked to this crosstalk. Sleep notably promotes declarative memory processing, and reward elements of food intake are often memorized via procedural memory systems.

The neurobiology of circadian rhythms provides the temporal organization of all basic aspects of an individual. Deregulation of circadian systems is linked to a broad variety of neurological disorders and other human conditions including obesity, anorexia nervosa, cardiovascular and mood disorders, and disturbances in memory and sleep. Further study on the neurobiology of circadian rhythms is needed to allow integration of different neurobiological disciplines and the establishment of circadian systems neurobiology (Fig. 3). One example of the advantage of this knowledge is the development of a ‘molecular-timetable method’ via blood analysis. This will lead to chronotherapy and personalized medication [64*], even more so when pushed forward to an organ-specific read out.

**References and recommended reading**

Papers of particular interest, published within the annual period of review, have been highlighted as:
- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 638).

Sleep and respiratory neurobiology


7 Essah-Parkin et al. described a dorsal medially positioned hypothalamic nucleus of Fuller et al. [5]. It shows that food anticipatory activity in mice does not depend on the DMH, and that it does not depend on the canonical, Bmal1 dependent, circadian molecular feedback loop.


14 This extensive study shows photopigmentation by red light in human pupil constriction to blue light. It provides action spectra for both test and potentiation stimuli, indicating the bi-stable nature of retinal melanopins that also provides the major light input to the SCN with bistable properties.


16 This study uses clock gene knockout mice (Cry1−/−; Cry2−/−) to show the importance of a functional retinal circadian clock for normal visual responses. Similar findings were obtained in retinal specific Bmal1−/− mice.


20 Devevelser S, Sembia K. Indirect projections from the suprachiasmatic nucleus to major arousal-promoting cell groups in rat: implications for the circadian control of behavioral state. Neuroscience 2008; 150:165–183.


24 This identifies a novel circadian output pathway of the SCN to VTA via MPON, linking circadian regulation of behavioral processes including arousal and motivation.


28 This study elegantly distinguishes between rewards derived from food anticipation versus rewards derived from food consumption, and is the first study to show this distinction in humans.


30 In this study the interaction between circadian rhythmicity, food intake and reward is nicely shown. It is a study that is strengthened by its elegant approach.

Demonstrates that several synaptic proteins increase during wakefulness but decline during sleep, which corroborates the synaptic downscaling hypothesis as a key function of sleep.


Demonstrates that the number of synaptic terminals decreases during sleep, and this decline was prevented by sleep deprivation, corroborating the synaptic downscaling hypothesis as a key function of sleep.


Interesting study on the relevance of extra-SCN rhythms for learning and memory performance.


The first study to show the role of the canonical circadian clockwork in time-place association in mammals. A role for cry genes in this type of associative learning and memory is demonstrated.


Per2 rhythms in amygdalar regions are coupled to the SCN rhythm, but can be uncoupled by homeostatic perturbations and hormonal states that directly influences motivated behavior.


It is shown that disruption of circadian rhythms selectively impairs emotional components of memory, linking circadian rhythm disturbances to mood disorders.


This review nicely addresses the various options by which memory training (learning) can act as a Zeitgeber, and how clock genes, circadian inputs and sleep/wakefulness may interact at the level of the hippocampus.


Interesting study with high potential clinical relevance.