The neurobiology of circadian rhythms
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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 accordingly. 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 many 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
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

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].
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 melanopsin containing retinal ganglion cells. Melanopsin knock-out mice (OPN4−/−) show disrupted sleep architecture [25–27] which might be explained by direct projections of melanopsin containing ganglion cells to the ventrolateral pre-optic 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−/− mice were found to lack the typical light/dark food intake pattern. Additionally, these Per2−/− mice do not develop food anticipatory behavior, whereas Per1−/− and wild type mice do [39]. Furthermore, Per2−/− 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. Deregulation 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 anticipation may be found 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−/− 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 [50,*], 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 detrimental to memory. For example, arrhythmic Siberian hamsters, delayed novel-object learning was impaired [52]. This suggests that hippocampal-dependent learning
Figure 3  Circadian control of activity, energy homeostasis and memory

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, gluing 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.

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References and recommended reading

Sleep and respiratory neurobiology


20 Essential and high quality study on health risks that can be induced in shift work and/or jet-lag causing circadian dis-entrainment.


24 This article is the first to show that obesity affects the circadian expression of clock genes in the central nervous system, rather than clock genes affecting metabolic state.


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.


Coordinated rhythmic expression of clock genes is found in the hippocampus, which is disordered in mice deficient for mPer1. The latter mice are severely impaired in hippocampus-dependent learning and memory, suggesting clock gene expression as a temporal structure needed for hippocampal learning and memory.


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.