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The hypothalamic integrator for circadian rhythms

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Although the suprachiasmatic nucleus (SCN) is well established as providing a genetically based clock for timing circadian rhythms, the mechanisms by which the timing signal is translated into circadian rhythms of behavior and underlying physiology have only recently come to light. The bulk of the SCN outflow terminates in a column of tissue that arches upward and backward from the SCN, and which includes the subparaventricular zone (SPZ) and the dorsomedial nucleus of the hypothalamus. Neurons within the dorsal SPZ are necessary for organizing circadian rhythms of body temperature, whereas neurons in the ventral SPZ are needed for circadian rhythms of sleep and waking. Ventral SPZ neurons in turn relay to the dorsomedial nucleus, which is crucial for producing circadian rhythms of sleep and waking, locomotor activity, feeding and corticosteroid production. This multistage processor provides the animal with flexibility so that environmental cues, such as food availability, ambient temperature and social interactions, can be integrated with the clock signal to sculpt an adaptive pattern of rhythmic daily activities that maximize the chances of survival and reproduction.

Introduction

It has been known for nearly half a century that large lesions of the mediobasal hypothalamus cause loss of circadian rhythms of locomotor activity, feeding and drinking [1] but the location of the biological clock was not pinned down until 1972. A key series of experiments in that year established that the suprachiasmatic nucleus (SCN) receives the bulk of the retinal input to the hypothalamus [2,3], and that lesions of the SCN cause loss of circadian rhythms [4,5]. Subsequent work showed that the individual neurons of the SCN contain a genetically driven clock mechanism, with a transcriptional–translational feedback loop that ensures a nearly 24 h cycle [6]. This cycle is then synchronized to the external light–dark cycle by input to the SCN from retinal ganglion cells that act as irradiance detectors (their slow responses are proportional to the light level) [7].

Although the events that control the SCN clock cycle have been delineated in considerable detail over the past decade, the mechanisms that convert that clock signal into patterning of a wide variety of physiological and behavioral rhythms have remained obscure. However, recent work has begun to identify the key pathways and neurotransmitters that are involved in this process. This review will focus on those mechanisms.

Output from the SCN

The projections from the SCN in rats were first shown by Swanson and Cowan in 1975 using autoradiographic tracing [8], and in more detail by Watts and colleagues in 1987 [9]. These same projections can be identified conveniently in sections through the SCN region that have been stained immunohistochemically for either arginine vasopressin (AVP) or vasoactive intestinal polypeptide (VIP), which are contained in many of the output neurons [10].

The SCN provides three major output pathways. One pathway runs dorsally and rostrally along the third ventricle, into the medial preoptic area and then up into the paraventricular nucleus of the thalamus. A second pathway runs caudally, along the base of the third ventricle, to the retrochiasmatic area and the capsule of the ventromedial nucleus. The third pathway, which contains by far the largest portion of the SCN efferent flow, travels in an arc dorsally and caudally. These axons give off numerous ramifications and terminals along their course through the regions just above the SCN (the ventral subparaventricular zone, or vSPZ) and just ventral to the paraventricular hypothalamic nucleus (the dorsal subparaventricular zone, or dSPZ). A smaller proportion of these axons then continue dorsocaudally into the dorsomedial nucleus of the hypothalamus where they terminate along its length.

Small numbers of SCN axons also directly innervate areas that are involved in regulation of feeding, wake–sleep cycles and secretion of hormones such as melatonin and corticotropin-releasing hormone (CRH). The projection to the dorsal parvicellular portion of the paraventricular nucleus is believed to activate neurons that send their axons to the intermediolateral column of the upper thoracic spinal cord, where they contact sympathetic preganglionic neurons that control pineal melatonin secretion [11,12]. This pathway is thought to be the major mechanism for regulation of melatonin cycles. Other projections containing just a few axons reach the CRH-producing cells in the paraventricular nucleus [11], the ventrolateral preoptic nucleus (which promotes sleep) [13] and the orexin-producing neurons in the lateral hypothalamic area (which are presumed to contribute to

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wakefulness) [14]. For reasons that will be discussed, these direct pathways probably are not sufficient to maintain circadian rhythms of corticosteroid secretion or sleep.

Studies in which donor SCN tissue is grafted into animals after lesions of the host SCN show reconstitution of circadian rhythms of some motor behaviors, despite the paucity or even lack of neural connections between the graft and the host brain [15,16]. These studies suggest that the SCN might release a diffusible factor (or factors) that can regulate circadian rhythms [17,18]. However, the grafts reconstitute only a portion of locomotor rhythmicity, and fail to restore rhythms of melatonin or corticosteroid secretion [19]. Thus, most SCN effects appear to rely on its synaptic targets [20], and even the paracrine effects of local diffusible factors must rely on nearby tissues that can relay the circadian signal.

Which SCN targets regulate circadian cycles of specific functions?

Early lesion studies of the circadian system primarily used methods that damage both neuronal cell bodies and axons passing through or near the lesion site (e.g. electrolytic lesions, mechanical lesions or colchicine injections). Unfortunately, the complex interweaving of cell groups and fiber pathways present in the hypothalamus made the results of such studies difficult to interpret. The roles played by SCN targets in circadian control of specific functions have been reassessed recently by using cell-specific toxins, in association with rigorous quantitative analysis of lesion effects. These toxins (e.g. ibotenic acid and other excitotoxins) kill only neurons whose cell bodies are at the site of the injection, and it is possible, by counting cells in the target nuclei and in the surrounding structures, to correlate the loss of neurons in each cell group with the behavior or physiological function that is being measured [21–23]. This approach avoids both false-negative errors (lesions that kill <70–80% of a target cell population can have small effects that are picked up on correlation analysis but are missed in comparing means of groups with and without lesions) and false-positive errors (finding an effect that is due to damage to adjacent cell groups).

Lu and colleagues placed injections of ibotenic acid along the outflow pathway from the SCN in rats, to determine the effects on circadian rhythms [23]. They found that lesions of the vSPZ caused >80–90% reductions in the amplitude of circadian rhythms of sleep–wakefulness and of spontaneous locomotor activity [23], and caused a similar loss of the circadian rhythm of corticosteroid secretion (J. Lu *et al.*, unpublished). The amplitude of the circadian rhythm of body temperature was reduced to a smaller degree, by $\sim$40%, and furthermore this reduction did not significantly correlate with vSPZ cell loss. Conversely, lesions of the dSPZ eliminated as much as 70% of the amplitude of the circadian rhythm of body temperature but had virtually no effect on rhythms of sleep [23] (Figure 1) or corticosteroid secretion. The dissociation of the circadian regulation of sleep and body temperature was surprising, because the two functions are generally very tightly correlated [24]. Melatonin

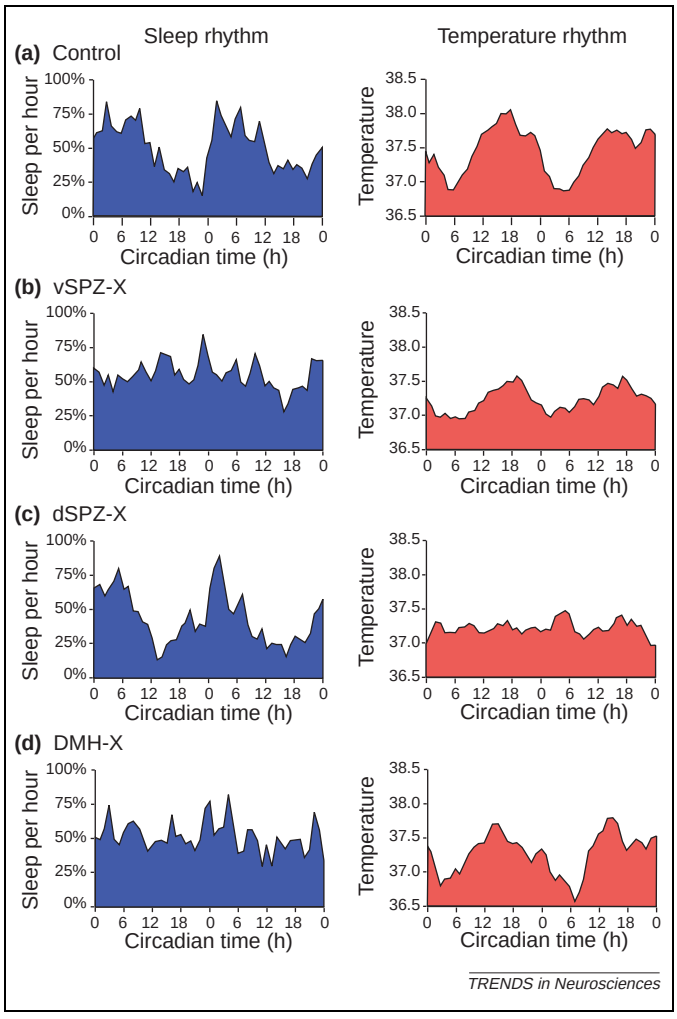

Figure 1. Differential regulation of sleep and body temperature rhythms by the hypothalamic circadian integrator. Total sleep and mean body temperature are plotted as a three-hour rolling average over a two-day period in a continuously dark environment for representative animals (data replotted from animals in Refs [22,23]). **(a)** Normal circadian pattern of sleep and body temperature. **(b)** After lesions of the ventral subparaventricular zone (vSPZ-X), most of the sleep rhythm is lost and the body temperature rhythm is reduced by $\sim$40%. **(c)** By contrast, lesions of the dorsal subparaventricular zone (dSPZ-X) have little effect on rhythms of sleep, but almost eliminate the circadian pattern of body temperature. **(d)** Lesions of the dorsomedial nucleus of the hypothalamus (DMH-X) cause profound loss of the circadian rhythm of sleep but have little effect on the rhythm of body temperature.

secretion was not affected by lesions of either the dSPZ or vSPZ (J. Lu *et al.*, unpublished).

Chou and colleagues then placed ibotenic acid lesions further downstream in other targets of the SCN and SPZ in rats [22]. These results demonstrated key sites for influencing circadian cycles and also eliminated other nearby sites as major participants. For example, lesions of the dorsomedial nucleus of the hypothalamus caused near total loss of the circadian rhythms of sleep–wakefulness, feeding, locomotor activity and corticosteroid secretion (Figure 1). The loss of rhythmicity was accompanied by a fall in the total amount of wakefulness (by one hour per day), and both locomotor activity and corticosteroid levels remained at the lowest level reached during the usual sleep cycle in intact rats. Body temperature showed only a modest reduction of the amplitude of circadian rhythm, but the mean was about half a degree lower. These results, which confirm earlier observations that electrolytic

lesions or muscimol injections into the dorsomedial nucleus of the hypothalamus impair corticosteroid rhythms [25,26], are consistent with the concept that this nucleus plays a crucial role in behavioral arousal [27].

Interestingly, the melatonin rhythm was not affected by dorsomedial nucleus lesions; nor did the loss of neurons in the regions adjacent to the dorsomedial nucleus alter circadian rhythms. Lesions of the ventromedial nucleus had little effect on the baseline mean or circadian rhythm of these functions (including feeding [28]). Lateral hypothalamic lesions, by contrast, reduced wakefulness by about one hour per day, but did not affect the circadian rhythm of wakefulness (although they did decrease the circadian rhythm of REM sleep). This would be consistent with the loss of orexin neurons, which are adjacent to the dorsomedial nucleus and are believed to have an important role in the regulation of REM sleep.

Anatomical studies indicate that the dorsomedial nucleus has extensive outputs to the major effector sites for these functions [22,29], compared with the SPZ and SCN [30]. Dorsomedial nucleus neurons innervate the ventrolateral preoptic nucleus, the lateral hypothalamic area and the paraventricular nucleus of the hypothalamus (Figure 2c,d). Furthermore, dorsomedial nucleus neurons innervating the ventrolateral preoptic nucleus were found to be predominantly GABAergic, whereas those innervating the lateral hypothalamic area primarily were glutamatergic or expressed thyrotropin-releasing hormone (TRH) [22]. This is consistent with the dorsomedial nucleus having a role in shaping circadian rhythms primarily by increasing wakefulness and arousal (exciting arousal neurons in the lateral hypothalamus and inhibiting sleep neurons in the ventrolateral preoptic nucleus). The dorsomedial nucleus is also the target for a major projection from the vSPZ [22,30].

Thus, the dorsomedial nucleus appears to sit at the terminal end of a column of tissue, beginning with the SCN and passing through the SPZ, which is crucial for maintaining circadian rhythms of most functions (Figure 2). The melatonin rhythm depends on a direct projection from the SCN to the paraventricular nucleus (Figure 2c), whereas body temperature rhythms are managed predominantly by a direct projection from the SCN to the dSPZ (Figure 2b). However, wake–sleep and locomotor rhythms (and probably also feeding and corticosteroid cycles) depend on two relays, one from the SCN to the vSPZ and a second from the vSPZ to the dorsomedial nucleus (Figure 2c,d). The dorsomedial nucleus, in this model, is the final common output site for a wide range of circadian rhythms.

Why have such a complicated, three-stage integrator?

This model for a hypothalamic circadian integrator allows the brain much more flexibility in sculpting circadian rhythms than would a simpler mechanism. For example, melatonin secretion, which is under the simplest type of monosynaptic regulation from the SCN to central effector neurons in the paraventricular nucleus, is hard-wired to the circadian clock in the SCN [31]. The SCN is more active during the light cycle, and its GABAergic neurons presumably inhibit the paraventricular premotor neurons that promote melatonin secretion [31]. During the dark cycle, this inhibition is released, and melatonin secretion peaks [22,27], regardless of whether animals are diurnal or nocturnal.

However, not all functions of animals can be adaptively regulated under such simple control. If the SCN is always most active during the light cycle [32,33], and the output neurons always bear the same phase relationship to wake–sleep cycles (i.e. the ventrolateral preoptic nucleus always is most active during sleep and the orexin neurons most active during waking), it would be difficult to explain why some animals are nocturnal and others diurnal. The multiple synaptic relays in the circuit allow an animal to adapt to evolutionary pressure by shaping a daily pattern of physiological and behavioral cycles that will be most adaptive to its ecological niche.

In fact, global patterns of behavior, such as nocturnal versus diurnal, are not fixed even within the life of many animals. Degus, for example, are South American rodents that are capable of inverting their daily cycle from diurnal to nocturnal [34]. In our laboratory, they were typically nocturnal when the ambient temperature was warm but diurnal when it was cooler [35]. This pattern is presumably adaptive (i.e. when it is warm at night, the animals can forage in the dark, where they are less likely to be attacked by predators that depend on visual recognition of prey; when it is cool at night, the loss of heat can outweigh the advantage, so the animals are active during the day). Similar patterns of activity have been recorded for Finnish bats (Figure 3a), which are often considered to be the quintessential nocturnal animals [36]. But during late spring and early fall, when nights are cooler and there are fewer insects flying to attract them (and fewer birds around to compete for the insects or to prey on the bats during the day), the bats are most active during the evening hours before the sun goes down, and can even become diurnal at the extremes of their season.

The multistage circadian integrator allows this flexibility because inputs from other systems, including thermoregulatory and feeding systems, can interact with the clock signal at synaptic relays in the SPZ and the dorsomedial nucleus to produce an optimally adaptive daily schedule. For example, both regions receive visceral afferent information from the parabrachial nucleus and information about the satiety hormone leptin, which has receptors in both the dorsomedial and ventromedial nuclei [37–39] (Figure 2). Receptors for other peptides believed to be involved in regulating feeding, such as cholecystokinin and ghrelin, are also found in some of these same structures [39,40]. Thus, the availability of food, a key component of survival, could have direct effects on the output of circadian patterns.

Experimental manipulation of circadian patterns by restricted feeding

It is possible to manipulate the circadian rhythms of a wide range of behaviors and physiological functions by restricting the timing of food availability during the day [41]. When rats, which are typically nocturnal, are allowed

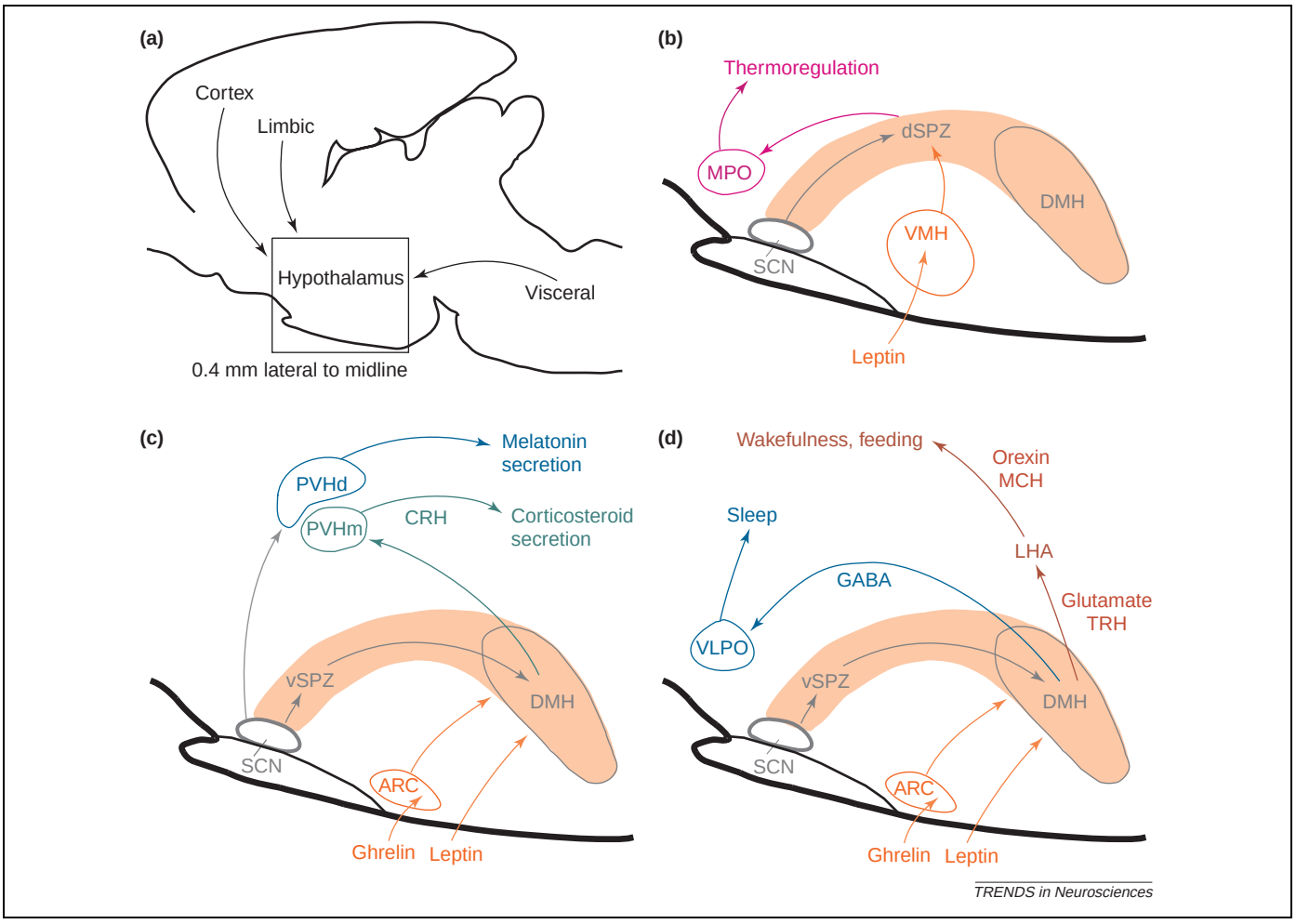

Figure 2. Summary of the CNS pathways that integrate the clock signal from the suprachiasmatic nucleus (SCN) with other cues to produce the circadian rhythms of physiology and behavior. **(a)** A sagittal drawing of the rat brain showing the area of the hypothalamus illustrated in panels (b–d). The hypothalamus receives three types of non-photic input that affect the regulation of circadian rhythms: cognitive input from the infralimbic, prelimbic and insular cortex; emotional input from the limbic system, including the hippocampus and amygdala; and visceral input from the nucleus of the solitary tract and parabrachial nucleus. **(b–d)** The SCN provides its most intense output to the subparaventricular zone (SPZ) and dorsomedial nucleus of the hypothalamus (DMH). Food-related cues from the hormones ghrelin and leptin (orange) are relayed from the arcuate nucleus (ARC) and ventromedial nucleus (VMH), in addition to impinging directly on the DMH and dorsal SPZ (dSPZ). (b) The dSPZ is crucial for controlling circadian rhythms of body temperature, through its projections to the medial preoptic region (MPO), which includes the median preoptic and ventromedial preoptic nuclei. (c) The SCN sends a projection to the dorsal parvicellular paraventricular nucleus (PVHd). PVHd neurons project to sympathetic preganglionic neurons in the spinal cord, which in turn regulate melatonin output by the pineal gland. The ventral SPZ (vSPZ) sends a relay to the DMH, which in turn controls a wide range of circadian responses, including corticosteroid secretion (c), and feeding and sleep–wake cycles (d). Outputs to the medial parvicellular paraventricular nucleus (PVHm) regulate the neurons containing corticotrophin releasing-hormone (CRH), which in turn control pituitary regulation of corticosteroid production. Projections from the DMH to the sleep-promoting ventrolateral preoptic nucleus (VLPO) are GABAergic, whereas outputs to orexin-producing and melanin-concentrating hormone (MCH)-producing neurons in the lateral hypothalamus (LHA) contain glutamate and thyrotropin-releasing hormone (TRH). The orexin-containing and MCH-containing LHA neurons are important for maintaining wakefulness and feeding, consistent with a role for the DMH in driving the arousal components of circadian cycles.

access to food only during the middle of the light cycle, they quickly adapt to eating during the day (Figure 3b). Interestingly, they become active about an hour before the food is actually presented and reduce locomotor activity during the dark cycle, thus shifting their activity rhythm. The peaks of body temperature (Figure 3c) and corticosteroid secretion also rapidly shift in coordination with the activity cycles.

There is evidence that this experimental paradigm, which mimics the exigencies faced by many animals in the real world, does not alter the underlying clock rhythm. The daily cycles of activity in the SCN remain locked to the light–dark cycle. If food access is changed back to *ad libitum*, the animals rapidly return to their original rhythm. Thus, the adaptability to external events (e.g. night-shift work or a midnight watch) does not necessarily depend on shifting the circadian clock in the SCN, but on adapting to alternative schedules that are shaped by the circadian integrator.

Recent studies have examined how such adaptation might work. Neurons in the dorsomedial nucleus show increased expression of the immediate-early gene *c-fos* during the dark period in rats that have *ad libitum* food access (J. Gooley and C.B. Saper, unpublished). When animals are placed on restricted feeding during the middle of the light period, the timing of greatest expression of Fos protein shifts to the day [42]. Thus, timing of the activation of outputs that shape the daily activity cycle of the animal by the dorsomedial nucleus is under regulation by the systems that monitor the availability of food. Such studies provide us with opportunities to understand the regulation of circadian rhythms better,

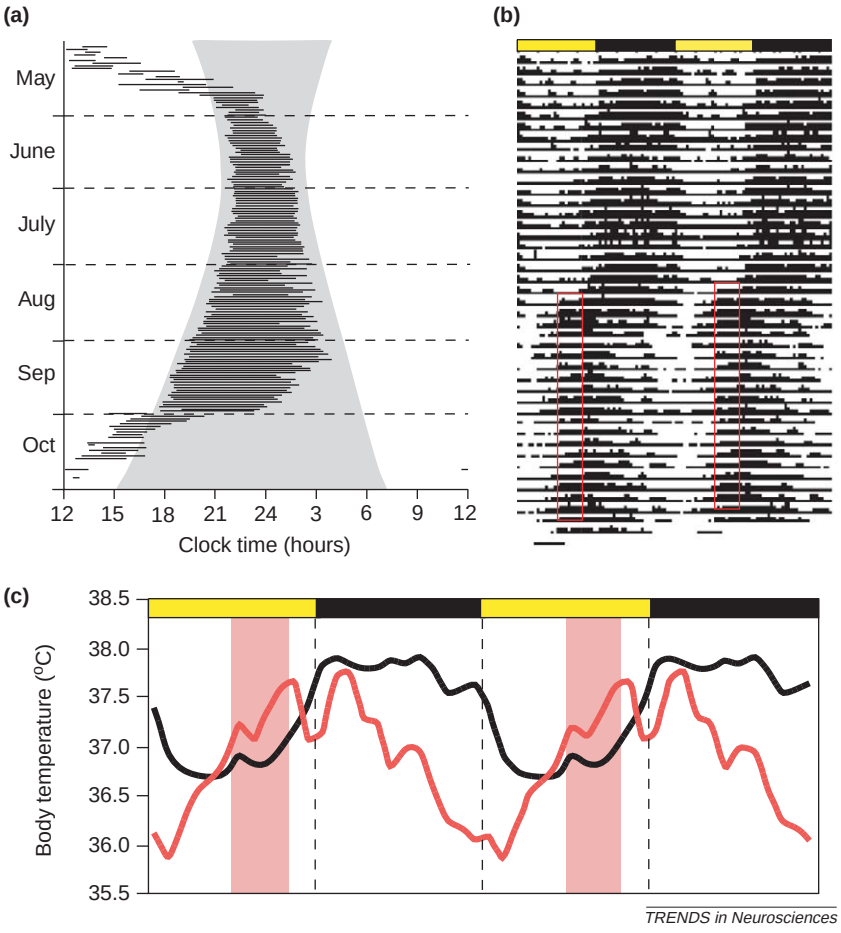

Figure 3. Circadian rhythms must be responsive to environmental stimuli. **(a)** Finnish bats are nocturnal mainly during the warm months of the summer. However, in the spring and fall, when environmental factors no longer favor night flight (fewer insects to eat during the colder nights, and fewer birds to compete with and to prey on bats during the day), their activity cycle shifts to the daylight hours. The gray region indicates the night period and the black bars show the actual activity time for the bats on each day. Modified from Refs [36,43]. **(b)** Similarly, in a laboratory environment, a rat given food only during the light hours will shift its activity cycle to coincide with food availability. In this double-plot raster graph of a representative animal, each line represents a 48-hour period, which is advanced by 24 hours for the next line. The yellow bar represents the light period and the black bar the dark period. Body temperature >36°C is plotted. At baseline, when food is available *ad libitum*, the onset of elevation of body temperature (which is closely tied to the activity cycle) is entrained to the onset of the dark cycle. After three weeks, food is restricted to a four-hour period (outlined by the red box), which is set from 1 pm to 5 pm (lights out at 7 pm). The activity and body temperature cycles shift by approximately five hours, so that there is a rise in both measures during the hours just before the food is present (anticipation) and a fall 12 hours later, even though this is now only half-way through the dark period. **(c)** To demonstrate that the body temperature changes are not due to the thermal activity associated with eating, on the last two days no food was given. However, the same anticipation is seen, as are body temperatures increases during the period when food had been present (highlighted in pale red). The black trace indicates the body temperature during *ad libitum* feeding and the red trace during the second day of food deprivation after entrainment to restricted feeding (this avoids any masking effects, such as an increase in body temperature due to the thermal effects of feeding behavior).

by defining the sites at which specific environmental stimuli (e.g. food availability) can reset the circadian integrator.

Summary

Within the past few years, the outline of the hypothalamic circadian integrator has finally begun to emerge. We now recognize that different functions can be controlled directly by the SCN clock (e.g. release of melatonin), or can be regulated by systems that are one synaptic relay (e.g. body temperature) or two synaptic relays (e.g. and feeding, locomotor activity, wake–sleep cycles and corticosteroid secretion) from the clock. The role of this complex integrator is now understood as allowing animals to respond adaptively to changes in their circumstances, such as alterations in ambient temperature and food availability (which are perhaps the two largest challenges for small rodents). In other words, nocturnal and diurnal behavior patterns can be shaped by circumstances to produce the activity pattern with the greatest adaptive value for the individual animal.

Determining inputs from cognitive systems that allow control of schedules in humans remains an important goal. By understanding the circuitry of the integrator, we could help individuals such as shift workers, sailors and those on night watches to adapt to the needs of their positions, and minimize fatigue and injury due to lapses of vigilance.

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