



## SYMPOSIUM

### Re-examining “Temporal Niche”

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**Synopsis** The circadian system temporally organizes physiology and behavior throughout the 24-h day. At the core of this organization lies a network of multiple circadian oscillators located within the central nervous system as well as in virtually every peripheral organ. These oscillators define a 24-h temporal landscape of mutually interacting circadian rhythms that is known as the temporal niche of a species. This temporal niche is constituted by the collective phases of all biological rhythms emerging from this multi-oscillatory system. We review evidence showing that under different environmental conditions, this system can adopt different harmonic configurations. Thus, the classic chronobiological approach of searching for “the” circadian phase of an animal—typically by studying circadian rhythms of locomotor activity—represents a narrow look into the circadian system of an animal. We propose that the study of hormonal rhythms may lead to a more insightful assessment of a species’ temporal niche.

#### Temporal niche

The circadian system regulates daily oscillations in physiology and behavior, defining a 24-h temporal landscape of mutually interacting biological rhythms across and within organisms. The “temporal niche” of a species is defined as the conjunction of these 24-h rhythms, whose timing is characterized by two basic parameters, the period and the phase. Under normal conditions, the circadian system is entrained by 24-h environmental cycles—such as the light–dark (LD) cycle—and the period of circadian rhythms is 24 h. Thus, the critical parameter that determines the adaptive value of rhythms is their phase. The typical approach to determine the temporal niche of a species has been to measure locomotor activity, either in the field or under laboratory conditions. Locomotor activity can be easily monitored and can typify an animal as diurnal, nocturnal, or crepuscular. Furthermore, in most animals, locomotor activity rhythms may represent a reliable phase-marker of that animal’s master circadian clock. Nevertheless, this deference to locomotor activity as the definitive output of the master clock can give a false impression of the

true temporal niche of a species for several reasons. First, decades of research have shown that rhythmic locomotor activity can be easily “masked” by both external environmental factors and internal physiological conditions. Masking acutely inhibits or stimulates rhythmic locomotor activity and hides the true phase of the driving clock. Second, the devices we use to monitor locomotor activity—running wheels, infrared detectors, or wrist actimeters—tell us nothing about the intention of increased locomotor activity and therefore of its adaptive value; for instance, similar levels of activity could be used to seek a mate or to escape from a predator. Third, the discovery of oscillators outside the suprachiasmatic nucleus (SCN), which houses the master circadian clock in mammals, and particularly of peripheral oscillators with remarkable autonomy from the master clock, clearly indicates that rhythmic locomotor activity gives us a very narrow view of the circadian systems operating within an animal. In fact, the discovery of peripheral oscillators challenges the concept of “the” circadian phase of an animal, as it is clear that the phase of a given circadian output is influenced by the

relative phases of many contributing oscillators. Finally, under natural conditions, different stable states for this multi-oscillatory system can emerge under different environmental conditions. Here, we examine counterexamples to the idea that locomotor activity represents a reliable and unified phase marker and propose that a look into endocrine rhythms may represent a more insightful way of revealing the temporal niche of a species.

### Looking for the phase of flies, rodents, and monkeys

There are two popular methods for determining the temporal niche of a species. The first is to observe when animals are active in their wild habitat. Locomotor activity during the day or night, respectively, typifies the species as diurnal or nocturnal, whereas activity during dawn and dusk makes the species crepuscular. As a follow up to this first method, animals are studied in the laboratory under a controlled LD cycle to assess their temporal profile of activity across the 24-h day. This second method allows the removal of environmental factors that may interfere with expression of the “true” circadian phase of the animal in the wild. However, when field and laboratory data conflict substantially, arguments can arise over which pattern more truly represents the phase of the animal. This may actually represent a false dichotomy brought about by the assumption that locomotor activity is a faithful expression of an animal’s concerted rhythms. Several examples clearly illustrate this point.

Recent findings investigating the locomotor activity rhythm of *Drosophila melanogaster* under “more natural” conditions find that there is a large bout of activity in the middle of the day, and that temperature cycles may shape daily locomotor activity more strongly than does photoperiod under these conditions (Vanin et al. 2012; Menegazzi et al. 2013). These findings fly in the face of libraries of laboratory work that suggested that flies were crepuscular, with bouts of locomotor activity preceding the lights on/off or off/on transitions. They may raise challenging questions about the true phase of *D. melanogaster*, but most importantly, they cast doubt on using locomotor activity to define the temporal niche of the animal. For example, in the newly described activity patterns, the fly has a morning and an evening bout of activity—both presumably coupled to morning and evening oscillators within the fly’s brain—as well as an afternoon bout of activity. Although the neural basis for the afternoon peak of activity remains unknown, the emergence of this afternoon

activity peak under more natural conditions or under the appropriate temperature cycles raises the question of what the true phase of flies is. Furthermore, it makes us wonder whether the phase of the fly’s clock, located in the small lateral neurons of its brain, differs between the laboratory and the more natural conditions. Finally, the emergence of a new bout of activity under different environmental conditions suggests this afternoon activity may have different functions than do evening and morning activities. The adaptive value of these locomotor activity bouts can obviously not be revealed by simply measuring infrared beam interruptions within an activity monitor 3 cm-long, particularly considering that in the wild *D. melanogaster* can migrate up to 16 km (Coyne et al. 1982, 1987).

Syrian hamsters tell a similar story in which rhythmic locomotor output differs between laboratory and wild conditions. In a standard LD cycle in a laboratory, hamsters are active during the dark phase, displaying nocturnality. In the wild, however, female hamsters are crepuscular and display no nocturnal activity (Gattermann et al. 2008). Similar discrepancies between activity profiles in the laboratory and in the wild have been found in the golden spiny mouse (Levy et al. 2007). These differences have been attributed to masking of the output of the circadian clock by natural stimuli that are absent in the laboratory. In “nocturnal” rodents like the hamster, there is an unparalleled localization of function within the SCN. In rodents, these bilaterally paired nuclei contain a circadian pacemaker that regulates all physiological and behavioral circadian rhythms (Klein et al. 1991; Welsh et al. 2010). The hamster itself has been a classic experimental model to demonstrate the master circadian regulation of locomotor activity by the SCN. SCN transplants into SCN-lesioned hamsters rescue locomotor activity rhythms with a genetically determined, donor-specific period (Ralph et al. 1990). Furthermore, the output of locomotor activity can be clearly linked to the pattern of clock gene expression within SCN neurons, even in split hamsters in which the antiphase oscillation of the left and right SCN leads to the expression of two antiphase bouts of locomotor activity (de la Iglesia et al. 2000). The temporal pattern of SCN clock gene expression, which represents the true phase of the core circadian oscillator, is unknown for hamsters in the wild. Nevertheless, it may conceivably be the same as in hamsters under an artificial LD cycle in the laboratory. What can we make of this changed relationship between SCN phase and the output of rhythmic locomotor activity? Are hamsters not intrinsically nocturnal or is their true nocturnality

never manifest in their desert homes? If this is the case, the SCN phase does not represent a good proxy for the animals' "true" phase. Even if activity patterns in wild hamsters reflected the SCN phase in a yet unknown pattern of gene expression, the question remains: what are the phases of the other rhythms that are regulated by the SCN pacemaker? For instance, are the phases of release of melatonin and glucocorticoids, which respectively track the night and the beginning of the active phase, similar in laboratory and wild hamsters? Is the timing of the surge of luteinizing hormone (LH)—typically regulated by the SCN in synchrony with locomotor activity—similar between the nocturnal laboratory female hamster and the diurnal desert female?

Nile grass rats (*Arvicanthis niloticus*) are diurnal in the field and under most laboratory conditions (McElhinny et al. 1997; Blanchong and Smale 2000). However, when housed with running-wheels, some individuals adopt a strongly nocturnal locomotor activity pattern while others remain day-active (Blanchong et al. 1999). In the night-active chronotypes, daytime behavioral sleep increases but several key features of the diurnal sleep-wake pattern are retained (Schwartz and Smale 2005), suggesting that these individuals are not "fully nocturnal" despite their strong preference for wheel-running at night. The SCN and neighboring ventral subparaventricular zone, which represents a relay station for SCN efferents to the rest of the brain (Moore et al. 2002), retain the rhythmic activity characteristic of day-active grass rats as measured by immunoreactivity for Fos protein (Schwartz and Smale 2005), and the clock proteins PER1 and PER2 (Ramanathan et al. 2010). By contrast, activation of Fos in hypocretin neurons (Nixon and Smale 2004), which are essential to sustain wakefulness and PER1/PER2 expression in the limbic forebrain, amygdala, and hippocampus (Ramanathan et al. 2010), which are essential for neural processing of complex tasks such as responses to reward, fear, and learning, adopt temporal profiles more similar to those of nocturnal species like the rat. Thus, adoption of a temporally reversed locomotor activity pattern in grass rats is associated with a radical, but incomplete, shift in physiological and molecular rhythms, leading to an individual that exhibits both "diurnal" and "nocturnal" characteristics.

A truly striking and illustrative example of the difference between behavior in the laboratory and behavior in the wild comes from the South American Owl Monkey *Aotus azarai*. In the laboratory under an LD cycle with dim light during the dark phase, these animals are nocturnal, which sets them apart

from other species of monkey (reviewed by Erkert 2008). Recent work using actimeters in the wild for 6–18 months at a time reveals a much more complex pattern than simple nocturnality (Fernandez-Duque et al. 2010; Erkert et al. 2012). Like hamsters in the wild, owl monkeys are typically crepuscular, with peaks of locomotor activity at dawn and dusk. Interestingly, on moonlit nights, the bulk of locomotor activity occurs during the night, making the animals appear nocturnal. On dark nights around the new moon, in contrast, the bulk of activity occurs during the day, making the animals appear more diurnal. This rhythmic switch from diurnality to nocturnality predictably repeats every 28 days with the lunar cycle. Although we lack data on endocrine and SCN-gene expression that could help track other rhythms, it seems unlikely that the SCN of this animal will exhibit both daily and lunar-monthly rhythms, or that all other circadian behavioral and physiological outputs will follow the same switching between nocturnality and diurnality. In fact, lunar eclipses have demonstrated that nocturnal activity during full-moon depends on the availability of moonlight (Fernandez-Duque et al. 2010). Regardless of the neural basis of these diel switches in activity, it is difficult to say whether owl monkeys in their home environment are "truly" diurnal, crepuscular, or nocturnal. Clearly, we cannot draw a complete temporal niche from the temporal locomotor activity pattern of these animals, since physiological rhythms such as those governing core body temperature or release of melatonin are not likely to be regulated in the same way as locomotor activity when the animals switch from nocturnality to diurnality.

Alternation between nocturnality and diurnality may be more common in nature than suggested by laboratory studies, in which clear and stable patterns of nocturnality and diurnality emerge (reviewed by Hut et al. 2012). In many cases, as in owl monkeys, these switches emerge from the masking effects of nocturnal light—indeed moonlight affects activity in several species (reviewed by Kronfeld-Schor et al. 2013)—or the activity cycle is altered by other environmental factors such as temperature, as it is also the case in owl monkeys (Fernandez-Duque et al. 2010). On the other hand, more complex ecological factors can determine these switches. For instance, the golden spiny mouse (*Acomys russatus*) can be found living with *Acomys cahirinus*, a dominant competitor. When the competitor is abundant, golden spiny mice forage during the day. When their competitors are absent, they forage by night. These and other intraspecific and interspecific interactions have been shown to shape the temporal niche

of different species (reviewed by Castillo-Ruiz et al. 2012).

In summary, it is clear that the timing of rhythmic behavior in nature is rather plastic, and this plasticity likely has adaptive value. In other words, it may be more adaptive for the golden spiny mouse to forage during the day if its competitor is present, or for owl monkeys to forage during the day as nights of new-moon approach, even though these species would otherwise show nocturnal activity patterns. However, the tradeoff associated with these switches in temporal niche cannot be assessed solely by monitoring locomotor activity, and a comprehensive ethological and ecophysiological approach is needed to determine the costs of displaying activity at different times of the day. The physiological basis for the plasticity of behavioral patterns is unknown; the evidence we review below, though, suggests that when animals switch from one temporal behavioral program to another, their multi-oscillator circadian system does not move cohesively to the new temporal niche. Instead, each new temporal niche may be associated with a specific ensemble of circadian physiological oscillations.

### **A circadian system of multiple oscillators**

The discovery first in *Drosophila* (Plautz et al. 1997) and then in mammals (Balsalobre et al. 1998) that cells in virtually every tissue outside of the brain contained a canonical molecular clock suggested for the first time that the organization of the circadian system was less dominant than previously thought (reviewed by Mohawk et al. 2012). The absolute command by the master circadian pacemaker within the SCN was challenged by the appearance of circadian oscillators in the most diverse areas of the periphery; these oscillators were in some cases as robust as the SCN itself and this suggested that they could indeed have some level of autonomy. Studies using jet lag protocols clearly demonstrated that the circadian oscillators within the SCN, the liver, the lung, and the skeletal muscle re-entrained with different speeds to the abrupt shift of the LD cycle (Yamazaki et al. 2000). Thus, jet lag led to transient states of circadian desynchrony, in which the oscillators throughout the body lost their normal phase relationships. This transient loss of harmony is likely not restricted to the periphery; similar protocols for jetlag lead to a transient desynchrony in the timing of behavioral states such as rapid-eye movement sleep and slow-wave sleep (Lee et al. 2009).

These experiments clearly show that at least transiently, there is not “a” circadian phase that can be identified for these animals. It also suggests that peripheral oscillators could be differentially responsive to other entraining environmental cycles, also known as “Zeitgebers.” Direct proof for this idea emerged from studies in the rat that showed that temporal restriction of food during the light phase leads to entrainment of a circadian oscillator in the liver, but not of the master clock in the SCN, leading to a new state of internal synchrony when compared with animals that feed during the night (Stokkan et al. 2001). Interestingly, entrainment of the liver but not of the SCN oscillator is associated with anticipatory locomotor activity just before the scheduled mealtime, yielding a new behavioral temporal program that apparently relies less on clock gene expression within the SCN, and that is associated with a new multi-oscillator configuration. Importantly, this configuration does not emerge from an unlikely ecological scenario such as jet lag, but rather from one that animals are indeed likely to encounter in the wild—that of access to food restricted to a time of the day when the animal is naturally inactive. Together, experiments both on jet lag and on restricted access to food indicate that specific environmental conditions demand different ensembles of central and peripheral oscillators and truly challenge the concept of “the” circadian phase of an animal; this phase is not only difficult to track, but it may not even be clear what it is meant by a “single phase.” These results also challenge the authority of the SCN as a master pacemaker and show that at least some of the “slave” oscillators in the periphery may have a significant level of autonomy.

The difficulty of identifying a single, unifying circadian phase may be particularly common in humans living in industrialized societies. Even when they may not be exposed to jet lag, they are typically exposed to “social jet lag,” namely the misalignment between our biological clock and our self-imposed sleep–wake cycle (Wittmann et al. 2006; Roenneberg et al. 2007). Our sociocultural schedules expose us to new internal circadian configurations that were likely absent in our ancestors. An extreme case of social jetlag is that of nocturnal shiftwork, which is associated with diseases such as obesity, cancer, cardiovascular disease, gastrointestinal disease, reproductive irregularities, and mental illness (Knutsson 2003; Flo et al. 2012). One hypothesis for this increased risk is that the various oscillatory systems cannot find a stable and normal phase relationship between themselves. Most nocturnal shift workers are not “truly” nocturnal. In other words,



their nocturnal activity has a phase relationship with other daily and circadian oscillations that is very different from this relationship when they work during the day. Even if we could track the phase of the master clock within the SCN in either a nocturnally or a diurnally active human being, this would tell us very little about the individual phases of all circadian oscillators. In other words, the rhythms that constitute our temporal niche, whether we work during the day or during the night, are not all represented by the SCN.

### The temporal internal environment

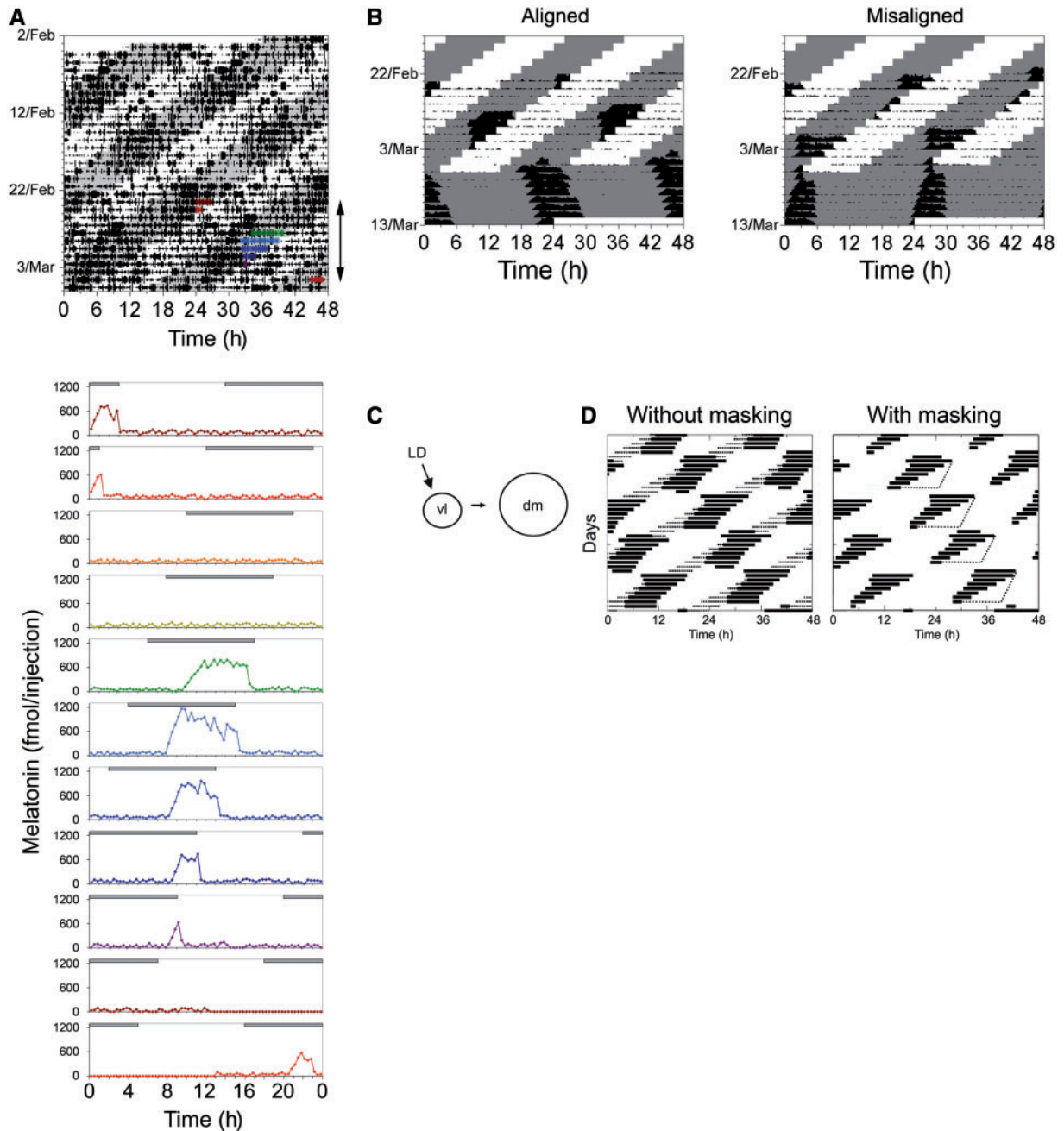
The studies reviewed so far show that locomotor activity not only can give a misleading view of the phase of the master circadian clock but also provide a poor view of the phases of multiple physiological rhythms that constitute the temporal niche of an organism. Indeed, the first formal studies of circadian rhythms in humans clearly revealed that spontaneous locomotor activity represented an unreliable marker of the phase of the master circadian clock (Aschoff 1965), and more recent studies have established that physiological rhythms, and specifically endocrine rhythms, represent a more faithful readout of the master circadian clock (Klerman et al. 2002). Here, we discuss the utility of examining hormonal rhythms to provide a more accurate picture of the internal phase(s) of an animal and to assess the meaning of specific peaks of locomotor activity. Much of what we know about this topic comes from studies investigating the circadian regulation of the release of melatonin and of the hypothalamic-pituitary-adrenal (HPA) and hypothalamic-pituitary-gonadal (HPG) axes.

The pineal hormone melatonin is robustly rhythmic, exhibiting a rapid 150-fold increase from nearly undetectable basal levels in the daytime to its nighttime peak. This nocturnal phase position of melatonin release is maintained both in day-active and in night-active species (Garidou et al. 2002; Challet 2007), and partially because of this, melatonin is frequently used as the most reliable marker of the circadian phase of the master circadian clock, particularly in humans (Klerman et al. 2002). The daily melatonin profile results from circadian control of the synthetic enzyme AANAT via a well-characterized multisynaptic pathway and the rapid inhibition of synthesis by light (Ganguly et al. 2002). The SCN is critical for both of these regulatory processes (Moore and Klein 1974; Klein and Moore 1979), although the mechanisms underlying the masking response are still not well understood.

We studied the SCN control of these two responses using a rodent model of forced internal desynchrony (de la Iglesia et al. 2004). Rats exposed to an 11:11 LD cycle (LD22) exhibit two locomotor activity bouts with differing periods: one bout is entrained to the 22-h LD cycle while the other bout is dissociated from it and shows a period of  $\sim 25$  h. As these two bouts move in and out of phase with each other, the animals transition between days of phase alignment in which both activity bouts occur in the 11-h scotophase to days of misalignment in which the dissociated bout occurs nearly entirely in the 11-h photophase. These 22-h and  $\sim 25$ -h bouts of activity parallel clock gene expression rhythms within two subdivisions, the ventrolateral (vl) and dorsomedial (dm), of the SCN, respectively, suggesting that the 22-h LD cycle has “pulled apart” two subregional circadian oscillators within the SCN. Using this model, we discovered that these vl and dm subregional oscillators contribute in distinct and separable ways to the photic masking and entrainment of the melatonin rhythm.

Pineal melatonin release under LD22 presents a single daily peak that loosely tracks the LD cycle but fails to fully entrain to it (Fig. 1A) (Schwartz et al. 2009), a phenomenon known as relative coordination (Pittendrigh and Daan 1976a, 1976b). The daily offset of melatonin release remains tightly coupled to lights-on, but the onset fails to phase-lock, gradually compressing the melatonin peak as the animal transitions from aligned to misaligned phase. At maximum misalignment of phases, the peak disappears entirely, then reappears 1–2 days later with a long delay in phase that maintains its nocturnal phase position and restarts the pattern. This single “zigzag” pattern of daily melatonin release parallels both rhythmic locomotor bouts—the rhythm entrained to the advancing LD cycle and the delaying dissociated rhythm. Furthermore, the compression and phase-jumping pattern persists when desynchronized rats are released into constant darkness (DD) at maximally misaligned phases (Fig. 1B), suggesting that these patterns are mediated via changes in parameters of the pacemaker and not solely via photic masking.

Based on these results, we generated a mathematical model of the desynchronized SCN in the forced desynchronized rat (Fig. 1C; Schwartz et al. 2009). Under simulated 22-h LD cycles, the vlSCN entrains to the LD cycle, but full entrainment of the dmSCN is prevented by weak coupling between vlSCN and dmSCN combined with the dmSCN longer intrinsic period. The resulting output pattern, with the addition of masking of melatonin release during the light



**Fig. 1** Forced desynchronization of the circadian release of melatonin reveals two subregional oscillators within the SCN. **(A)** Double-plotted actogram (upper panel) showing locomotor activity under LD22 and the days at which pineal melatonin was sampled via microdialysis (arrows). Colored bars represent the daily melatonin peaks; these peaks are single-plotted below (note: Colors appear only online, not in the printed journal.). The color of each profile corresponds to the color of each bout of melatonin in the actogram above; gray lines at the top of each profile indicate the dark phase. **(B)** Representative double-plotted actograms showing profiles of melatonin release (black bars) observed after release into DD. An animal released into DD during a day of alignment (left) shows a melatonin peak of long duration, whereas an animal released into DD during a day of misalignment (right) shows a melatonin peak of short duration. **(C)** Schematic diagram of decoding of light by SCN subregions. **(D)** Simulation of the coupled dmSCN (thick lines) and vlSCN (thin lines) is shown in **(C)**. The vl oscillator is entrained to LD22, whereas the dm oscillator is in relative coordination (left panel); synthesis of melatonin is controlled by the dm oscillator. When photic masking of the release of melatonin (right panel, dotted lines) is added to the model, the resulting profiles closely resemble observed profiles of melatonin release. Figures adapted from Schwartz et al (2009).

phase, closely resembles the experimental melatonin profiles seen in forced desynchronized rats (Fig. 1D; Schwartz et al. 2009). Together, these data indicate first that the dmSCN controls the circadian melatonin profile and second that the vlSCN is an oscillator that can both acutely inhibit melatonin release and entrain the dmSCN oscillator. Of note, our observations in the forced desynchronized rat clearly indicate that melatonin release is a much more accurate marker of the phase of the SCN clock than locomotor activity (Liu and Borjigin 2005; Schwartz et al. 2009). However, on days of misalignment, neuronal oscillators within the SCN lack a cohesive phase, and the daily melatonin onset—considered in isolation from duration and amplitude of the peak, and from other behavioral rhythms—provides little or no information about the “true” phase of the master clock. Our study also underscores the utility of mathematical models to more accurately reveal the phase of the master circadian pacemaker; mathematical tools have been classically used to unmask the phase of the human circadian pacemaker (Kronauer et al. 2007) but they can clearly be exploited to extract other circadian parameters such as period and amplitude (Leise et al. 2013). Mathematical modeling will likely be critical for extracting information on phase from time series that can be inherently noisy or limited, as in data from field studies. In addition, modeling may enable isolation of multiple oscillators in animals that are not 100% internally synchronized, enabling a more complete understanding of how different clocks drive independent outputs.

In subsequent experiments, we supported this hypothesis by showing that the response to phase-shifting nocturnal pulses of light is compartmentalized in the vlSCN and dmSCN of forced desynchronized rats (Schwartz et al. 2010): the vlSCN upregulates *Per1* expression in response to light independently of the dmSCN phase, whereas behavioral phase shifts only occur when the dmSCN and vlSCN are in phase with each other. Thus, the subregional oscillators in the vlSCN and dmSCN exhibit distinct functional roles, and can act somewhat independently in regulating their own phase and output. However, observed behavioral and physiological markers of rhythmicity (i.e., the “hands of the clock”) reflect the aggregate activity of both subregions.

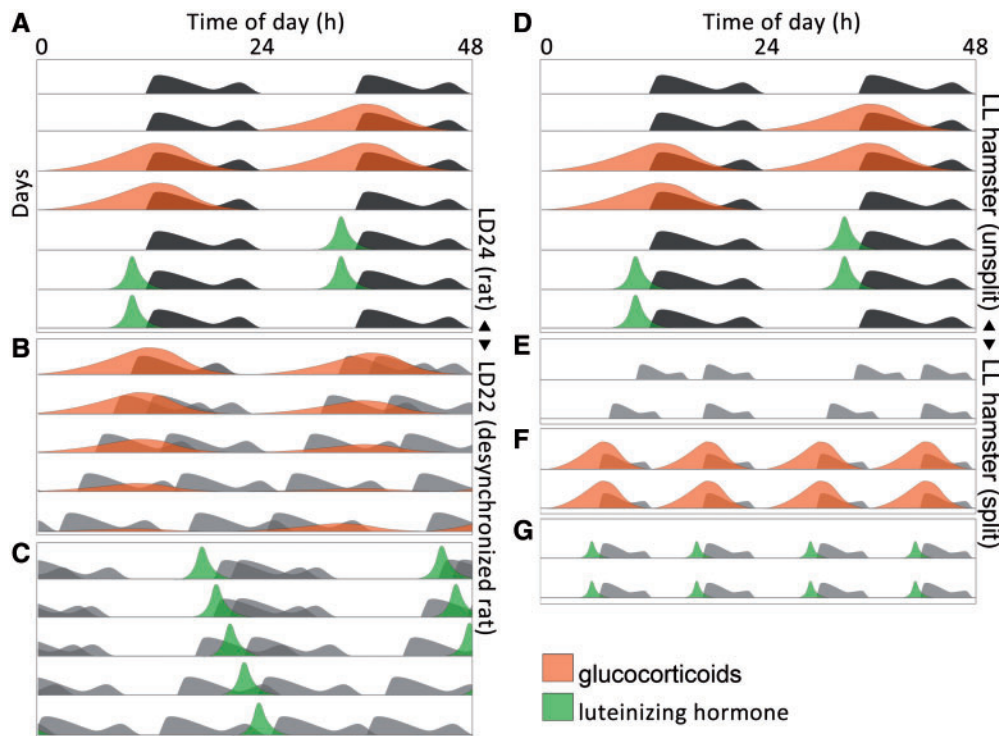
Under 12:12 LD laboratory cycles, plasma glucocorticoids, the end products of activation of the HPA axis, vary throughout the day with the peak occurring at about the time of increased activity (i.e., morning for humans and evening for nocturnal animals, like rats and hamsters) and the nadir

occurring at about the time of decreased activity (Dunn et al. 1972). Although the SCN-dependent nature of this glucocorticoid rhythm was established many years ago (Moore and Eichler 1972; Szafarczyk et al. 1979), how this physiological rhythm correlates with imposed changes in locomotor activity has only been demonstrated recently. Two animal models that have shed light on this topic are the split hamster and the forced desynchronized rat. As described above, splitting in hamsters results in two bouts of wheel-running activity that are stably coupled about 12 h apart. In this model, we have shown that two peaks of plasma cortisol, the primary glucocorticoid in hamsters, are phased with the onsets of each bout (Lilley et al. 2012). Interestingly, there are no concomitant peaks in plasma adrenocorticotropic hormone, the primary secretagogue for cortisol in split hamsters, suggesting that other factors drive glucocorticoid secretion under these conditions.

In the forced desynchronized rat, during days of alignment, corticosterone (the primary glucocorticoid in rats) peaks at about the onset of locomotor activity as in LD24-housed animals. However, during days of misalignment, when the animal exhibits two out-of-phase bouts of locomotor activity, the peak of the corticosterone rhythm is blunted and does not track the onset of either locomotor activity bout (Wotus et al. 2013). Similar findings were observed in human models of desynchrony (Scheer et al. 2009).

In the case of the female HPG axis, the surge in LH that triggers ovulation, and occurs every day in an ovariectomized estrogen-treated female, correlates with the two bouts of locomotor activity in split hamsters, much like cortisol (Swann and Turek 1985), and this bimodal surge of LH is correlated with the alternate activation of left- or right-side GnRH cells (de la Iglesia et al. 2003). During days of misalignment in the desynchronized rat, the timing of the LH surge, unlike corticosterone, does correlate with locomotor activity; however, it is tightly linked to the dmSCN-associated bout of activity, but not to the vlSCN-associated bout (Smarr et al. 2012).

Here, we have two rodent models that produce two distinct bouts of activity, yet exhibit very different hormonal profiles (Fig. 2). In the case of behavioral splitting, cortisol and LH correlate with both bouts. However, in the case of desynchrony during misaligned days, cortisol and LH are not only uncoupled from both bouts but each shows their own distinctive, yet predictable, patterns of secretion. Although these similar behavioral profiles—associated



**Fig. 2** Diagram of double-plotted rat (A–C) and hamster (D–G) locomotor activity (dark-gray raised areas) across the day. Stereotypic patterns of release of glucocorticoids (orange) and LH (green, in an ovariectomized female treated with estrogen) are overlaid to allow phase comparisons between the rhythms of locomotion and the rhythmic activity of the HPA and HPG axes (note: Colors appear only online, not in the printed journal). Rats switched from LD 12:12 (A) to LD:11:11 (black arrowheads) show continuously varying, yet predictable, overlap between two different locomotor rhythms, with a right-drifting component (period ~25 h) tied to the dmSCN and a left-drifting component entrained to the 22-h LD cycle. Stereotyped days from highly overlapping (aligned) to minimally overlapping (misaligned) days show the changing phase relationship of corticosterone (B) and LH (C) to bouts of locomotor activity. Hamsters exposed to LL but unsplit show normal circadian profiles of cortisol and LH (D). Spontaneous splitting of the locomotor output (E) is associated with splitting of both hormonal rhythms (F, G). Note that days of misalignment and splitting both show two bouts of locomotor activity per day, but radically different hormonal profiles.

with clearly unique endocrine profiles—emerge from very artificial manipulations of the LD cycle, they clearly demonstrate the importance of examining physiological rhythms before drawing conclusions about an animal’s temporal niche. They also point to the fact that overt locomotor activity displayed in a laboratory cage may reflect very different behavioral entities. For instance, while the split female hamster shows two bouts of activity that each follow the release of an ovulatory signal from the brain—suggesting that both bouts are associated with behaviors related to reproduction—the misaligned desynchronized rat shows two bouts, only one of which is correlated with an ovulatory signal.

### A circadian system regulated by every oscillator

Rhythmic locomotor activity is an easily monitored behavior, both in the field and in the laboratory, and in many cases it may represent a good readout of the

phase of the master circadian clock. Nevertheless, defining the temporal niche of an animal or a species, based solely on this behavioral maker, may be misleading. Even when the phase of the master circadian clock may be assessed accurately, the multi-oscillatory nature of the circadian system, and the symphony of circadian rhythms it defines, demands a more comprehensive definition of temporal niche. In addition, the experimental evidence indicates that this temporal niche may adopt different configurations depending on environmental demands. In other words, a specific array of internal phase relationships among the circadian oscillators of an animal may be normal under specific environmental conditions but abnormal under others. Although activity-based characterizations of temporal niches as nocturnal, diurnal, crepuscular, etc. are useful, similar phenotypes of rhythmic locomotor activity may arise from very different physiological rhythms configurations. The adaptive value of a specific temporal



program of behavior will be determined to a great extent by the underlying temporal organization of physiological processes.

Although the SCN was classically seen as the master oscillator and sole regulator of circadian rhythms, the discovery of peripheral oscillators challenged its hierarchy. The SCN remains a central circadian pacemaker with a unique localization of function. Nevertheless, its top hierarchical position in the multi-oscillatory circadian system can become subordinate to a peripheral oscillator under specific ecophysiological conditions. Understanding how the SCN and peripheral oscillators regulate each other will be critical to assess how humans respond to circadian challenges and how animals cope with changing ecological factors. Endocrine studies like the examples given above, as well as studies of tissue-specific genomic and proteomic outputs will likely build a more comprehensive view of the circadian system—in which the interactions between multiple circadian oscillators are incorporated—and a more nuanced and precise view of temporal niches.

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