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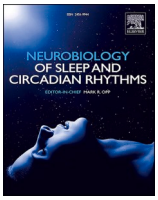

# Sleep and the circadian system: The latest gossip on a tumultuous long-term relationship

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## 1. Love, not at first sight

Any visitor to our planet would register that human activity is drastically reduced during the nighttime, and that during a large portion of the night we fall into an unconscious and largely inanimate state we call sleep. One would think that this blatant, seemingly inescapable nightly intrusion in overt behavior, which occurs with a strikingly regular rhythm, should have represented the first chapter of a long-lasting intimate relationship between two apparent soulmates: the biological clock that times our 24-h biological rhythms, and sleep. However, the story of this couple is far more complex.

By the mid-20th century, scientists who studied 24-h rest-activity rhythms were occupied trying to sort out whether these rhythms were generated by endogenous timing mechanisms or instead represented a simple response to environmental cycles. Once it was clear that indeed a ~24-h autonomous biological oscillation—a circadian clock— was responsible for these rhythms, the effort shifted to finding this clock and elucidating how it was put together. Meanwhile, the discovery of the electroencephalogram as the first tool to "see" the human mind at work mesmerized those interested in the secret messages of the sleeping brain, and led to a rather fanatical classification of cortical activity—noisily reported through the skull and scalp—into long and short waves of different amplitudes that correlated with different behavioral states and types of sleep. While circadian researchers were interested in dissecting parts of the clock, determining how light interacted with these parts to entrain it, and disassembling and putting it back together, exploring whether the clock sustained rhythms of sleep or sleep stages was a less attractive endeavor for them. Similarly, sleep researchers were interested in why it was so difficult to wake somebody up from "slow-wave" sleep vs. a sleep stage with an electrical signature resembling wakefulness, or whether either of these stages were associated with dreaming and learning, but showed very little interest in how sleep was temporally organized throughout the day.

## 2. First kiss

The sleep-circadian relationship moved from one with little mutual interest to one resembling two teenagers too shy to ask the other to dance. Before they left the party, though, they finally worked up the nerve to chat in the early 1980's. The thorough analysis of long-term polysomnographically recorded sleep in humans isolated from temporal cues revealed that the timing of sleep, and especially of rapid-eye-movement (REM) sleep, were under strong circadian regulation. However, it was not easy to convince the sleep field to join the dance. Indeed, the general belief was that the main driver of human sleep timing was merely a homeostatic demand for sleep. Simply put, we stay awake until we are tired enough to fall asleep, and then sleep manifests itself as the sleep debt is paid; non-REM slow-wave sleep is abundant at the beginning of the night when the sleep debt is high, and less so at the end when the debt is low, at which time REM sleep becomes more prevalent. In contrast to this view, the studies by Czeisler and collaborators, done first under spontaneous circadian desynchrony and later under forced-desynchrony conditions, provided irrefutable evidence for the circadian regulation of sleep and, more specifically, of REM sleep (Czeisler et al., 1980a,b). These studies showed that when the rest-activity cycle was scheduled outside of the limits of entrainment several physiological

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outputs continued to display a circadian rhythm with a period longer than 24 h. Under these conditions, when scheduled rest coincided with the circadian temperature minimum, sleep propensity, and particularly REM sleep propensity, where maximal.

## 3. Enchantment

Thankfully, this awkward initial encounter was followed by a healthy relationship that lasted several decades. Borbély's quintessential two-process model of sleep regulation was the first blessing of the union, providing both a powerful visual representation of the two fundamental forces regulating sleep timing and the progression of sleep stages, as well as quantitative predictive power for real-life sleep scenarios (Borbely, 1982; Borbely et al., 2016). The two-process model found further sophistication in studies that showed that lesions of the suprachiasmatic nucleus (SCN), the hypothalamic center that timed other behavioral and circadian rhythms, also abolished the circadian timing of sleep. In squirrel monkeys, these lesions also increased overall sleep, suggesting that the role of the C process—enacted by the SCN—was to induce wakefulness and oppose the S process in its homeostatic drive to sleep (Edgar et al., 1993). This so-called *opponent process*, however, did not apply to other species, in which SCN lesions disrupted circadian regulation of sleep but did not change its overall amount (Mistlberger, 2005). Meanwhile, the circuitry connecting the SCN and sleep-regulatory centers began to be mapped (Scammell et al., 2017). Later studies unmasked the circadian timing of REM sleep in nocturnal rodents, showing that, as in humans, REM propensity peaked during the circadian temperature minimum, and pointing to the first putative subregions of the SCN regulating REM sleep timing (Cambras et al., 2007; Lee et al., 2009). Multiple studies highlighted the unavoidable impact of the circadian temperature minimum—a reliable marker of the lowest circadian wakefulness phase—on nocturnal workers (Wyatt et al., 1999). A renewal of the vows occurred when clock gene mutations identified in *Drosophila* in the early 1970's paved the way for our understanding of the genetic basis for several prominent sleep disorders in humans (Konopka and Benzer, 1971; Ashbrook et al., 2020). Indeed, the decades-long relationship reached the point at which circadian timing became a defining feature of sleep for virtually all animals.

Sadly, however, this relationship has since developed a bit of an on-again, off-again nature. Long-term sleep studies in humans continued beyond the classic studies but have become scarcer and cost prohibitive in recent years. Similar studies in animals are rare; indeed, only a handful of labs focused on sleep are interested in its circadian regulation and similarly very few circadian labs are dedicated to understanding sleep as an output of the circadian clock, despite the fact that it is arguably the output with the most prominent physiological and behavioral consequences.

Individually, each field has made remarkable strides. The understanding of sleep's functions, despite the fact we have not nailed "the" function, has increased drastically, and it has become a common subject not only in clinics but also among policy makers, school superintendents and union leaders, to name a few. On the other hand, the current understanding of the circadian clockwork has not only been rewarded with the Nobel Prize but also left the laboratory. Lessons from circadian biology impact not just the clinic but also when and what we eat, how we use artificial lights and how we travel across time zones. Yet despite these individual successes, the synergism of the circadian-sleep couple needs new perspectives to thrive.

## 4. Disenchantment

While the 40-year-old relationship has remained largely healthy, like in any partnership, there have been setbacks. A significant rate-limiting factor has been the difficulty of recording polysomnographic sleep long-term in animal models. What is relatively non-invasive in humans—electrodes attached to the scalp for electroencephalographic (EEG) recordings—is highly invasive in animals, in which electrocorticographic (ECoG) electrodes need to be implanted through the skull. These cumbersome implants are typically tethered through wires to amplifiers, rendering them prone to electrical noise, short-lasting and disruptive of normal behavior. These limitations have precluded long-term experiments that are essential to unmask circadian properties. In fact, with the exception of the early courtship days of the circadian-sleep love affair and a few later encounters, studies that record sleep continuously in laboratory rodents for more than 2–3 days are rare (Cambras et al., 2007; Lee et al., 2009; Sanchez et al., 2019; Richardson et al., 1985). The availability of new minimally invasive long-lasting wireless ECoG implants will likely facilitate long-term (weeks to months) recordings.

A second limitation of long-lasting polysomnographic sleep recordings is that EEG (or ECoG) recordings are typically scored by an experienced researcher who divides them into 5-10-sec bins and patiently scores bin after bin. A 1-month study with 10 individuals produces a few million bins to be scored. This limitation has been recently circumvented by new sleep-stage scoring algorithms that can identify the signatures of each sleep stage with reliability similar to a human operator. This and similar advances have made long-term polysomnographic recordings in multiple animals a reality (Allocca et al., 2019; Sanchez et al., 2019; Caldart et al., 2020).

A third problem has been the availability of animal models that display large amounts of REM—or paradoxical—sleep. This is a limitation not just for sleep research as a whole but specifically for the circadian-sleep interface, particularly if we aim to gain understanding of the circuitry and neurochemical basis of the signature circadian regulation of REM sleep.

A fourth fundamental limitation has been the lack of easily accessible diurnal models. Those of us studying the mechanisms by which the circadian system regulates sleep are focused on animals that sleep during the day and are awake at night. While we may learn a lot about sleep in nocturnal animals, the use of these animal models to further our understanding of circadian regulation in humans represents a major constraint that may greatly mislead us.

## 5. Couple's therapy

Any decades-long partnership needs fresh mutual sources of inspiration; if we want to keep the relationship healthy we need to think proactively about what will rekindle the spark.

The study of circadian rhythms and of behavioral sleep in *Drosophila* has been pivotal for our understanding of the circadian regulation of sleep as well as the discovery of signals that homeostatically regulate sleep (Toda et al., 2019). However, it is unlikely that these invertebrate models will reveal much about many important aspects of sleep regulation. The identification of neuronal circuits involved in cortical arousal, the differential dependence of slow-wave sleep and REM sleep on circadian regulation, or the changing architecture of sleep cycles throughout the night, to name a few, are clearly research questions that demand mammalian models. There is a critical need for reliable animal models that allow for a combination of sleep-stage recordings, cell type-specific genetic mutations and the mapping and manipulation of neural circuits. Mice meet all of these criteria, but rekindling the spark in the circadian-sleep partnership may demand models that are more compelling. As new bioengineering tools become available and potentially allow for the development of these capabilities in unconventional vertebrates, we may need to go back to the comparative physiologist mindset and develop animal models that are appropriate to answer questions that are at the core of the sleep-circadian relationship (Rattenborg et al., 2017). An alternative is to continue capitalizing on the unique transgenic advantages that the mouse offers but exploit newly emerging naturalistic conditions that induce diurnality, such as the work-for-food paradigm (Riede et al., 2017). No matter what animal model the circadian-sleep partnership uses to understand sleep in the diurnal brain, we should not forget that the timing of sleep has real

behavioral and physiological tradeoffs under natural conditions, and that not all diurnality or nocturnality is created equal (Smarr et al., 2013). Thus, it is critical that the life history of the model species always remains a central framework in our attempts to understand sleep function and regulation.

Technology development is critical to expand our understanding of how the circadian system regulates sleep and specific sleep stages, and how sleep in turn talks back to the clock. Until now the study of vertebrate sleep has been made possible by our ability to assess cortical activity through EEG and ECoG. But these two fundamental tools of sleep physiologists also represent the narrowest bottle necks for progress in sleep-circadian studies. Technologies that allow for the non-invasive long-term recording of sleep in the brain or its subregions in freely behaving animals, are desperately needed. It may be time to move beyond electrodes and leverage imaging strategies, which are starting to unmask sleep properties in novel sleep animal models (Leung et al., 2019). Other approaches have used detailed behavioral video tracking to reliably distinguish sleep stages in mice (Fisher et al., 2012; McShane et al., 2012). Newer approaches to mapping brain activity in freely behaving animals can be paired with the exponential increase in the ability of machine-learning algorithms to detect biologically relevant patterns. All we need are signatures of sleep stages; after all, slow waves were an obscure epiphenomenon of the deepest sleep until we discovered how they were generated.

Systems neuroscience has reached a remarkable level of sophistication with our ability to optogeneticallly or chemogenetically affect the activity of specific brain regions or even specific neurons within a region. We can now manipulate the behavioral state of a mouse by either stimulating or inhibiting specific neural circuits, and switching between sleep and wake, or between non-REM and REM sleep, is no exception (Shiromani and Peever, 2017). These strategies will be critical to maintain healthy progress at the sleep-circadian interface. Lesion studies in which SCN ablation leads to arrhythmicity in the timing of sleep can be misleading. For instance, the absence of 24-h-organized sleep in SCN-lesioned animals could be an indirect consequence of arhythmic locomotor activity. Similarly the absence of 24-h rhythms in REM sleep in an SCN-lesioned animal could be the consequence of arhythmic sleep and not proof of direct regulation of REM timing by the circadian system, a role that has only been unmasked through internal desynchrony models. We now know that the SCN is even more heterogeneous than previously thought, and that different neuronal subtypes of the SCN likely play unique roles in maintaining specific circadian rhythms and sensing the internal and external environment (Bussi et al., 2020; Wen et al., 2020). Targeting these specific neuronal groups for excitation and inhibition within the SCN, along with their respective targets, will represent a key strategy to truly understand the mutual interactions between the circadian system and sleep centers. Indeed, these strategies are beginning to be used to reveal the role of specific neurons in the central pacemaker directly regulating sleep (Collins et al., 2020). Conversely, transcriptomic and proteomic profiling at the regional or single-cell level represents a powerful tool to understand how sleep influences the circadian system both within and outside of the SCN. For instance, sleep deprivation can modify clock gene expression in the brain and peripheral tissues (Curie et al., 2015), but it remains to be determined in which cell types this occurs in and what is the impact of this change on gene expression. Importantly, these omics approaches are starting to disentangle circadian from sleep regulation. For instance, whereas the 24-h oscillation of most synaptic transcripts is under circadian regulation regardless of sleep state, the 24-h oscillation of synaptic proteins and their phosphorylation state depend on the alternation of sleep states and not on circadian time (Bruning et al., 2019; Noya et al., 2019).

These approaches need to be further exploited to address fundamental unanswered questions: Why do some species need remarkably less daily sleep than others? What are the mechanisms behind consolidated vs. highly fragmented sleep in different species? How does a similarly ticking central circadian clock promote sleep at completely different times of the day in diurnal vs. nocturnal species? How is sleep regulated in species that naturally shift between diurnality and nocturnality. What are the mechanisms by which the SCN times REM sleep? What is the neural basis of internal desynchronization of sleep stages under circadian desynchronization protocols? What are the biological consequences of the misaligned sleep architecture that results from challenges to the circadian system such as shift work, jet lag and the later chronotypes that artificial light has promoted? How do the circadian and homeostatic processes interact to sculpt the architecture of each sleep cycle? Although the study of sleep and of the circadian system are partners naturally meant for each other it is important we continue to address these and newly emerging questions to nurture the healthy evolution of the partnership.

## CRediT authorship contribution statement

**Raymond E.A. Sanchez:** Writing - original draft. **Horacio O. de la Iglesia:** Writing - original draft.

## Declaration of competing interest

No conflict of interest to declare.

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