Investigating sleep homeostasis using an unusual instability

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ONE OF THE CORNERSTONES of our present knowledge about sleep in mammals is that sleep is regulated by homeostatic mechanisms. The basic concept of sleep homeostasis is relatively simple: as waking duration increases, sleep pressure increases, and sleep intensity during subsequent sleep is a function of prior waking duration. The intensity of sleep is reflected by the activity of the slow waves (~1–4 Hz) of the electroencephalogram during non-rapid eye movement (NREM) sleep (2).

The concept of sleep homeostasis explains, despite this relatively simple approach, a significant portion of the variation observed in the amount of sleep and in the activity of the slow waves during NREM sleep. In many mammalian species it was shown that the amount of slow-wave activity in NREM sleep depends on the duration of prior wakefulness, and, in the human (1), rat (11), and mouse (10, 12), mathematical models simulating the sleep homeostatic response have been applied successfully.

One of the difficulties in the analysis of sleep homeostasis is the other major player in the game of sleep regulation: the circadian clock. The circadian clock, which resides in the suprachiasmatic nuclei (SCN) of the hypothalamus, clearly influences the timing of sleep and wakefulness. In addition, it seems to continuously influence sleep propensity by supporting or inhibiting sleep at the appropriate time of day (8, 9). Recently, it was shown that SCN neuronal activity is responsive to changes in sleep and electroencephalogram slow-wave activity (5). It is therefore no surprise that many models of sleep regulation include the influence of the circadian clock (2).

Although circadian and sleep homeostatic mechanisms both influence the occurrence of sleep, it is generally assumed that homeostatic and circadian sleep regulatory processes function independently (2). This assumption is supported by the findings that homeostatic responses in sleep persist after circadian rhythmicity has been abolished by SCN lesion (14, 19, 21) and that the circadian process can be manipulated by light in the morning without changing NREM sleep slow-wave activity (7).

To separate the influence of sleep homeostasis and the circadian clock, many different protocols and animal models have been applied. This ranged from forced desynchrony protocols (8), using arrhythmic species such as the guinea pig (20), SCN lesions (14, 19, 21), Siberian hamsters adapting to winter photoperiod (4), or the loss of circadian gene function (15, 22). All have their own specific drawbacks, ranging from problems adhering to the protocol, uncontrollable damage through the lesions, compensation of gene deficiency during development, or lack of an appropriate control group.

Larkin et al. (15a) in this issue of the American Journal of Physiology-Regulatory, Integrative and Comparative Physiology have been able to investigate sleep homeostasis in the Siberian hamster (Phodopus sungorus) without interference of the circadian component. Their approach is unique in that they were able to do this in intact hamsters under normal light-dark conditions. The Siberian hamster is a nocturnal rodent that is used in many physiological studies because it displays a large spectrum of behavioral and physiological adaptations when photoperiod changes. One of the adaptations to short (winter) photoperiod is the occurrence of daily torpor during which body temperature drops to ~15–20°C for several hours during the rest phase of the animal. To investigate the influence of torpor on sleep was the reason why we introduced this species to the sleep community approximately 10 years ago (3).

After we started working with the Siberian hamster, I visited the group of Gerard Heldmaier in Marburg, Germany. Stephan Steinlechner, who was in Marburg at that time, showed rather interesting, but also disturbing, data. He was able to render the hamsters arrhythmic just by applying light pulses during two consecutive nights, as if the animals had a very labile circadian pacemaker. At that time we considered this to be a serious warning not to make one single mistake with the light-dark cycle, because animals would be rendered useless for the experiments we had planned. Ruby and coworkers apparently discovered the phenomenon independently. In their protocol the animals became arrhythmic after phase delaying the light-dark cycle by 5 h. Both groups published their results in the following years (17, 18).

In this issue, Larkin et al. from the Ruby lab make use of this anomaly. They considered that because the animals become arrhythmic, it should be possible to investigate sleep homeostasis without interference from the circadian clock. They show that the differences between the rhythmic and arrhythmic animals did not disappear in continuous darkness confirming that the arrhythmicity is not caused by a sudden blindness of the arrhythmic animals. Establishing this, they were able to study the effects of sleep deprivation in anatomically and genetically intact hamsters that nevertheless lacked overt circadian rhythmicity. They compared sleep regulation in these animals with hamsters that underwent the same phase delay protocol but remained rhythmic. The results show that circadian processes are of major importance controlling the daily distribution and consolidation of sleep and wakefulness, but also confirm the prevailing notion that circadian processes do not substantially contribute to, or modify, sleep homeostasis.

In light of this result, a remarkable finding of the Larkin study is the increase in the amount of sleep over 24 h in the arrhythmic animals to daytime values of the rhythmic animals. In addition, NREM sleep in arrhythmic animals was more consolidated and they were more difficult to keep awake during the sleep deprivations than rhythmic control animals. These observations seem to indicate that sleep pressure is increased in the arrhythmic animals independent of prior waking duration. Squirrel monkeys rendered arrhythmic by SCN lesions also show an increase in total sleep time, suggesting that an SCN-dependent process facilitates the initiation and maintenance of wakefulness and opposes sleep pressure during the active...
phase of the animal (9). However, in previous studies in rodents (rats and mice), lesioning the SCN was never accompanied by an overall increase in sleep duration to daytime values (13, 14, 19, 21). Sleep in the Siberian hamster made arrhythmic by a phase shift of the light-dark cycle differs in that respect from sleep in other rodents made arrhythmic through SCN lesions. This is even more remarkable, because the total amount of sleep in the Siberian hamster does not change between winter and summer photoperiod. Even when the animals’ sleep loses circadian organization during the winter photoperiod and resembles sleep of the arrhythmic hamsters in the Larkin study, the total amount of sleep over 24 h is remarkably similar to the amount under summer physiology (6, 16). Because it is unknown how much an SCN-lesioned Siberian hamster sleeps, the question remains whether the result of the phase delay protocol really mimics the absence of the circadian clock. It may be that we are dealing with a different phenomenon possibly related to changes in output function of the clock. Larkin et al. rightfully remark that their results may also be an indication of a species difference between hamsters, mice, and rats. The latter is interesting in its own right and should be investigated further.

In a broader perspective, Larkin et al. clearly demonstrate that this animal model can be applied to the broad spectrum of research related to biological rhythms, particularly the coordination of circadian rhythms with other biological processes. The present paper investigating the coordination between sleep and circadian rhythms is a perfect example and hopefully many will follow.

REFERENCES