RESEARCH HIGHLIGHT

Biochemical pathways of sleep

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Kim et al. and Zhou et al. discover how a kinase signaling pathway activates numerous genes that regulate the time mice spend in deeper non-rapid eye movement sleep. This work provides new entry points into the molecular regulation of sleep.

The core functions of sleep are unknown.[1] Yet, when we are sleep deprived we have an increased need to sleep and we sleep longer to catch up on lost sleep. But how is the sleep need registered at the biochemical level, and could this give us clues as to the function of sleep? The sleep drive is frequently conceptualized as two separate drives (circadian and homeostatic) that independently wax and wane, additively determining when to sleep and for how long.[2] The circadian drive specifies when to sleep and wake over 24 h, but the sleep homeostat is hypothesized to provide additional control, tracking the amount of wakefulness so that sleep can be replenished if needed.[2] In mammals, the circadian drive is controlled by the suprachiasmatic nucleus in the hypothalamus. But what controls the sleep homeostat? This seems to involve multiple cell types, distributed throughout the mouse brain.[1] Also, the biochemical nature of the homeostat is a mystery. In mammals, the sleep homeostat is inferred to exist from changes in the delta power in the electroencephalogram (EEG). After sleep deprivation, the delta power of non-rapid eye movement (NREM) sleep increases, possibly reflecting an increased depth of sleep (Note: delta power, 1–4 Hz in the EEG, together with reduced muscle movement, is diagnostic of NREM sleep). There is also a catching up on lost NREM sleep after sleep deprivation. Why and how this happens is unclear, and was the basis for the work highlighted here.[3,4]

Insight into the factors regulating the amount of sleep came from an impressively large-scale genetic screen for genes that influenced sleep in mice. This screen identified the *Sleepy* gene.[5] *Sleepy* mice have more NREM sleep with higher delta power. *Sleepy* encodes a permanently active version of Salt-Inducible Kinase 3 (SIK3), a widely expressed serine-threonine protein kinase in the brain, but also found in many peripheral tissues.[6] The pathways regulating SIK proteins outside of the brain have been intensively studied because of their importance in controlling, for example, lipid metabolism, gluconeogenesis and tumor progression.[6] SIK kinases are activated by Liver Kinase B1 (LKB1), a master kinase, and inhibited by Protein Kinase A.[6] Downstream targets of SIKs include histone deacetylases (HDACs), such as HDAC4 and HDAC5 proteins, which when dephosphorylated, shuttle into the nucleus and associate with transcription factors such as CREB to repress or stimulate gene expression.[6] When SIK3 is activated by LKB1, SIK3 phosphorylates HDAC4 and HDAC5, and these are retained in the cytoplasm.

How does SIK3 control sleep amount and apparent depth? Two substantial and linked studies have now followed up with the initial work.[3,4] A further unbiased genetic screen revealed the *Sleepy2* gene; as with the *Sleepy* mutation, the *Sleepy2* mice have more NREM sleep per 24 h, with higher delta power.[3] *Sleepy2* is a mutation in the *Hdac4* gene, which makes much less HDAC4 protein.[3] Building on what is known about SIK3 signaling,[6] the two studies used both mouse crosses for conditional genetic manipulations,[3] or AAV transgene delivery,[4] to knock out components, or express mutant versions, of the LKB1-SIK3-HDAC4/5 pathway in different brain regions. For example, *Lkb1* knockouts in brain strongly reduced NREM sleep amount and delta power (the opposite effect of the *Sleepy* mutation, because LKB1 is required to activate SIK3), although the effect, for unknown reasons, only happened during the dark phase of the 24-h cycle.[4] Conversely, in adult brains, *Hdac4* and *Hdac5* conditional knockouts increased NREM sleep time and delta power.[4] Both studies revealed the same results, but stronger phenotypes arose with acute gene manipulation in adult mice compared to chronic manipulations obtained by mouse breeding, suggesting some compensatory factors.[3,4]

The model is that during wakefulness, HDAC4 and HDAC5, in association with the transcription factor CREB, hinder sleep-associated genes in glutamatergic neurons in the neocortex from being transcribed. As wakefulness progresses, more SIK3 becomes activated by phosphorylation by LKB1. Activated SIK3 phosphorylates HDAC4 and HDAC5, causing these proteins to be sequestered in the cytoplasm, allowing sleep-promoting genes to be activated. The LKB1-SIK3-HDAC4/5 pathway extends NREM sleep time by changing gene expression in neurons in the posterior hypothalamus, whereas activating the pathway in neocortex increases NREM delta power (recall the original *Sleepy* mice).[3,4] Ironically, in mice with gain-of-function mutations in *Hdac4* that force HDAC4 to stay in the cell nucleus, sleep homeostasis still takes place.[4] Therefore, the LKB1-SIK3-HDAC4/5 pathway is dispensable for regulating sleep need. Still to be determined is how, and whether, the LKB1-SIK3-HDAC4/5 pathway links with the other sleep drive, the circadian system. Others have found that mice with a SIK3 total depletion have circadian disruptions, and that one of the circadian clock proteins, PER2 was upregulated.[7] SIK3 is hypothesized to phosphorylate and destabilize PER2.[7]

A tacit assumption[2] is that increased delta power always means increased sleep. But delta power can often be uncoupled from sleep need, because there are many different routes to produce this power change. The LKB1-SIK3-HDAC4/5 pathway, by eliciting changes in gene expression, could alter NREM delta power by

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changing how the network generates synchronicity, but this may not have anything to do with sleep per se. For example, selective knockdown of inhibitory $GABA_A$ receptors in the reticular thalamic nucleus strongly enhances NREM delta power.[8] In this instance, the substrate/circuitry on which the "sleep algorithm" runs changes, but not the sleep drive itself. Similarly, manipulations of circuitry often produce changed sleep times. Selectively killing GABA neurons in the midbrain ventral tegmental area gives a severely reduced NREM-sleep-time phenotype, selective for the dark period, similar to that caused by deleting *Lkb1*.[4,9] There are many ways to break the system which give the same result. This may give a false impression of causality.

In spite of these caveats, the LKB1-SIK3-HDAC4/5 pathway's impact on sleep, although not unique to sleep, is an undeniably important discovery for the sleep field.[3,4] Could the other metabolic functions regulated by LKB1-SIK3-HDAC4/5 signaling be embedded in the function of sleep? Work on worms (*Caenorhabditis elegans*) concluded that a SIK3 kinase homologue, KIN-29, which like SIK3 phosphorylates and inhibits HDAC4, promotes both lipid mobilization and a sleep-like state (lethargus) just before the worm moults its exoskeleton.[10] Moulting is energy intensive. Sleeping could allow optimal use of lipid stores for the moult.[10] The mammalian SIK3 kinase pathway could similarly integrate metabolism and sleep. Understanding what precisely this involves could explain why we feel terrible after missing a night's sleep.

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ADDITIONAL INFORMATION

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