Reversible changes to neural proteins may explain sleep need

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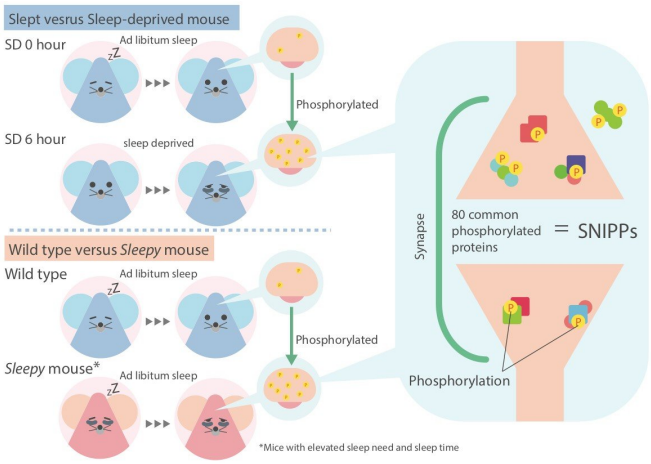

By comparing the brains of sleep-deprived mice and Sleepy mutant mice, the phosphorylation of 80 proteins, named SNIPPs (Sleep-Need-Index-Phosphoproteins), was found to be increased along with sleep need. Credit: University of Tsukuba

Long periods of waking can lead to cognitive impairment, and the need to sleep continues to build up. Sleep then refreshes the brain through alterations in molecular biochemistry. These changes impact neuronal plasticity and brain function, but the molecular underpinnings of "sleepiness" are not well understood.

Researchers at the International Institute for Integrative Sleep Medicine (WPI-IIIS) in Japan's University of Tsukuba went looking for the biochemical changes that form the basis of this sleep-wake cycle.

A current theory of the sleep-wake cycle suggests that waking encodes memories, whereas sleep consolidates memories and restores synaptic homeostasis. The researchers suspected the molecular substrate of sleepiness should be seen in all brain regions, and should accumulate gradually during waking and dissipate through sleep. Their findings, recently published in *Nature*, revealed that protein phosphorylation may be the key.

During normal function, cellular proteins may be modified by reversible addition of a chemical phosphoryl group, this is known as phosphorylation. The researchers used techniques for analyzing which proteins are phosphorylated and which are not. This enabled them to identify and quantify the phosphorylation of a wide range of brain proteins in sleep-deprived mice, and in mice with a single point mutation, named Sleepy, that increases both sleep time and sleep need.

"Protein functions can be switched on or off by site-specific phosphorylation, or modulated by cumulative phosphorylation of multiple sites," says study first author Zhiqiang Wang. "So it seemed likely that phosphorylation patterns may reveal the processes underpinning sleep need."

Immunochemical and mass spectrometry results showed increases in the number of protein phosphorylation in the whole brains of Sleepy mutant mice and normal sleep-deprived mice. Importantly, the abundance of proteins did not change and the researchers found the pattern of phosphorylation increase in Sleepy mutant mouse brains mimicked that of sleep-deprived mouse brains.

They also found a dose-dependent increase in the number of phosphorylation events in the whole-brain phosphoproteome, which tracked increasing sleep need.

By analyzing the quantity of change in phosphorylation, the researchers identified 80 proteins that are hyper-phosphorylated when the mouse is sleepy, which they termed the Sleep-Need-Index-PhosphoProteins (SNIPPs). The phospho-state of SNIPPs changed along with sleep

need. Importantly, the SNIPPs identified were predominantly synaptic proteins.

"By comparing sleep-deprived mice and Sleepy mutant mice, we were able to filter out the effects of prolonged waking, prolonged sleeping and stress," corresponding author Masashi Yanagisawa says. "Our findings show that the phosphorylation/dephosphorylation cycle of SNIPPs may be a major way that the brain regulates sleep-wake homeostasis."

The study provides evidence that prolonged wakefulness causes hyper-phosphorylation, whereas sleep promotes global dephosphorylation of the brain proteome. Given that the sleep-wake cycle impacts cognition, this research could aid in understanding sleep-wake patterns for optimal brain function.

More information: Zhiqiang Wang et al, Quantitative phosphoproteomic analysis of the molecular substrates of sleep need, *Nature* (2018). DOI: 10.1038/s41586-018-0218-8

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