Abstact

Sleep is a fundamental behavior observed across the animal kingdom, with no species capable of sustaining life without engaging in sleep or a sleep-like quiescent state. The sleep-wake cycle switch is controlled by a complex cascade of molecular and cellular processes, including neurotransmitter signaling, gene expression, and neuronal activity. As the wakeful state persists, the body's propensity to sleep increases, leading to an accumulation of “sleep need” that drives animals to engage in sleep behavior. This drive to sleep dissipates as the sleep need is satisfied. Although recent advances have led to the accumulation of information about executive neural circuitries regulating sleep and wake states, the molecular and cellular mechanisms that drive the switch remain largely unknown.

Our research group identified salt-inducible kinase 3 (SIK3) kinase as a key regulator of sleep homeostasis through the mouse forward genetics study. *Sleepy* splice mutant allele of *Sik3* led to a marked increase in total time and EEG delta power (1-4 Hz) during non-rapid eye movement sleep (NREMS). In an independent screening, a second hypersomnic mutant pedigree was discovered, with loss-of-function *Sleepy2* splice mutant alleles in *Hdac4*. The CRISPR/Cas9-driven gene-modified mice harboring *Hdac4* splice mutation (herein denoted as $Hdac4^{SA}$) also exhibited increased total time and delta power during NREMS, similar to the *Sleepy2* pedigree. In contrast, the phosphor-deficient HDAC4 mice of SIK3-targeted phosphorylation site, $Hdac4^{245A}$, showed an opposite trend with a decrease in the NREMS time and delta power. Additionally, somatic expression of $Hdac4^{245A}$ in $Sik3^{Sleepy}$ mice via CNS-transducible adeno-associated virus alleviated the hypersomnic phenotype of $Sik3^{Sleepy}$ mutants, suggesting a direct link between SIK3 and HDAC4 in NREMS regulation.

Furthermore, neural group and cell-type specific manipulations of the kinase-substrate pair using the Cre/loxP recombination system revealed distinct subsets of neurons that differentially regulate NREMS time and delta power. Manipulation of SIK3-HDAC4 in cortical excitatory neurons led to changes in delta power during NREMS, indicating its involvement in regulating sleep depth, whereas manipulation in the hypothalamic excitatory neurons was associated with changes in the NREMS time, or sleep quantity. The power of the delta frequency represents the level of synchronization between the cortical up- and down-state and is implicated in synaptic homeostasis. SIK3, through its downstream effectors, may regulate the transcriptional changes associated with synaptic plasticity, along with post-translational modifications in the excitatory synapses. Moreover, SIK3 signaling in the hypothalamic excitatory neurons may govern the switch between sleep-wake states and their maintenance.