論文の内容の要旨

論文題目 Understanding the role of $Ca^{2+}$-dependent hyperpolarization pathway in sleep/wake homeostasis.

（睡眠覚醒恒常性におけるカルシウム依存的過分極経路の役割の理解）

氏名　史　蕭逸

Sleep is a widely conserved and indispensable physiological phenomenon in animals. Although researchers investigated neuronal and biochemical nature of sleep regulation and revealed some sleep/wake-promoting nuclei and secreting substances, the detailed molecular mechanisms underlying the regulation of sleep duration and sleep homeostasis in mammals are still elusive. In this study, based on the prediction obtained from a simple computational model, which recapitulates the electrophysiological characteristics of the slow-wave sleep, we identified several genes whose knockout (KO) mice exhibits abnormal sleep duration by using the new high-throughput system for analyzing sleep mutants that mice exhibit abnormal sleep phenotypes. Sleep deprivation experiments and nonlinear dynamical analysis against mutants' phenotypes revealed the possibility that these mutant mice had impaired sleep homeostasis. Together, these results propose a hypothesis that $Ca^{2+}$-dependent hyperpolarization pathway underlies the regulation of sleep duration and sleep homeostasis in mammals.

Chapter 1 reviews the overview of modern sleep studies from a view of sleep duration and sleep homeostasis. I then introduce a computational prediction that a $Ca^{2+}$-dependent hyperpolarization pathway might play a role in generating the electrophysiological characteristics of the slow-wave sleep and hence in the regulation of sleep duration. Finally, I introduce a new developed high-throughput system for analyzing sleep mutants.

Chapter 2 focuses on the regulation of sleep duration and asks whether the impairment of the $Ca^{2+}$-dependent hyperpolarization pathway results in decreased sleep duration in mice. We generated and analyzed 21 lines of KO mice, revealing that knocking out $Ca^{2+}$-dependent K+ channels (*Kcnn2* and *Kcnn3*), voltage-gated $Ca^{2+}$ channels (*Cacna1g* and *Cacna1h*), or $Ca^{2+}$/calmodulin-dependent kinases (*Camk2a* and *Camk2b*) decrease sleep duration while knocking out plasma membrane $Ca^{2+}$ ATPase (*Atp2b3*) increases sleep duration. Pharmacological intervention validated that the inhibition of NMDA receptors decreases sleep duration.

Chapter 3 focuses on the regulation of sleep homeostasis and asks whether the impairment of the $Ca^{2+}$-dependent hyperpolarization pathway results in abnormal sleep homeostasis in mice. To understand the dynamical structure underlying the sleep homeostasis, I performed a series of nonlinear dynamical analysis, simplex projection, S-map, and convergent cross mapping, against a time series of Psw (the transition

probability between sleep to wakefulness, an index of wakefulness pressure) and Pws (the transition probability between wakefulness to sleep, an index of sleep pressure) in wild-type (WT) or mutant mice. The result suggested that the memory storage of wakefulness and sleep are impaired in these mutants. This possibility was partly validated by the abnormal response to sleep deprivation in *Kcnn2* KO mice.

Chapter 4 summarizes and concludes the results presented in this thesis and discusses the future experiments to understand the detailed molecular mechanisms underlying the regulation of sleep duration and sleep homeostasis.