School of Integrative and Global Majors
Ph.D. Program in Human Biology (HBP)

論文概要

Dissertation Abstract

Title of Doctor Dissertation: Genetic analyses of molecules and neural circuits regulating REM sleep
（レム睡眠の制御を担う分子や神経回路の同定と解析）

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Abstract

Rapid eye movement sleep (REM sleep) is a distinct physiological state observed in certain mammalian or avian animal species and is featured by desynchronized neocortical activity, sympathetic activation, and muscle atonia. While REM sleep is well known as a major source of dreaming, the physiologic roles of REM sleep and the neural substrates that contribute to generating REM sleep remain poorly understood. The sublaterodorsal nucleus (SLD), located in the mesopontine tegmental area, has been shown to have a crucial role in REM sleep regulation. However, the precise identity of the neurons critically involved remain unsolved.

In my doctoral course, I aimed to identify the molecular markers for neurons that regulate REM sleep and generate genetic tools that allow manipulation of REM sleep, which should enable analyses of the physiologic roles of REM sleep from a totally novel approach. To this end, I focused on two candidate genes, previously identified to be selectively expressed in the SLD, as potential markers of SLD neurons that regulate REM sleep in our laboratory. Here, I generated two *Cre*-KI mice to drive the expression of Cre recombinase in neurons expressing either of the candidate genes. In other words, I established *Cre*-KI mouse strains in which two distinct neuronal subpopulations in the SLD are genetically labeled by Cre expression. Analyses of these two mouse strains suggested that the two subpopulations are also distinct in terms of function, each subpopulation either negatively or positively regulating REM sleep. In the first *Cre*-KI mouse strain, when Cre-labeled neurons in SLD were activated using the DREADD system, the amount of REM sleep was drastically reduced, suggesting that these neurons negatively regulate REM sleep. By contrast, in the second *Cre*-KI mouse strain, when the Cre-labeled neurons were genetically ablated via Cre-dependent

DTA expression, the amount of REM sleep was strikingly diminished, suggesting that these neurons have a critical role in promoting REM sleep. Furthermore, when I analyzed the behavior of these mice with largely reduced REM sleep, they exhibited significantly reduced anxiety-like behaviors, detected by open field test and elevated plus maze test, and depression-like behaviors, detected by forced swim test, perhaps implicating a role for REM sleep in regulating emotion. Moreover, the homozygote of the first *Cre*-KI mice in which the protein product of the candidate gene was not detected, showed higher amount of REM sleep after cage changing, suggesting that the protein product of the candidate gene *per se* is involved the regulation of REM sleep under environmental stimuli.

Collectively, I genetically identified two distinct populations of neurons in the SLD that contribute to the bidirectional regulation of REM sleep. The results demonstrated that REM sleep could be essentially suppressed in a laboratory mouse for a significant period of time. I expect that this mouse model provides a starting point to study the fundamental function of REM sleep.

In addition, I found that a gene whose *in vivo* function was totally unknown, is involved in the regulation of REM sleep. Compared to the neuronal circuitry involved in sleep regulation, the molecular mechanism of sleep regulation is far less understood, and further analyses of the gene might provide novel insights about the molecular bases of REM sleep.