Form 2

Dissertation Abstract

Report no.	(Course-based) No.1028	Name	YADANAR MOE
Dissertation title	Analyses of Molecular Expression and Physiological Function of a Sexually Dimorphic Nucleus in the Hypothalamus (視床下部における性的二型核の分子発現と生理機能の解析)		

Abstract

※ The abstract should be in keeping with the structure of the dissertation (objective, statement of problem, investigation, conclusion) and should convey the substance of the dissertation.

Sexually differentiated brain contains the nuclei that exhibit morphological sex differences in volume, neuron number, distribution pattern of neurons, neurite length, and synaptic number. Such nuclei are called sexually dimorphic nuclei (SDNs) that are considered to regulate sex-specific or sex-biased physiological phenomena. The first SDN was discovered in the preoptic area of the hypothalamic brain in rats and termed the sexually dimorphic nucleus of the preoptic area. This SDN exhibits male-biased sex differences in volume and neuron number and is suggested to be involved in male sexual behavior. In addition, there are SDNs identified in the hypothalamus of many species. However, it is still the potential to uncover novel SDNs and their physiological roles. Recently, a novel SDN has been found in the dorsal hypothalamic area (hereafter SDN-DHA) in mice. The SDN-DHA is located in the region sandwiched between the principal nucleus of the bed nucleus of the stria terminalis (BNSTp) and calbindin-sexually dimorphic nucleus (CALB-SDN), both of which are male-biased SDNs and consist of calbindin neurons. In contrast to the BNSTp and CALB-SDN, the SDN-DHA exhibits female-biased sex differences in neuron number and estrogen receptor-α expression (ERα). However, the physiological functions and molecular basis of the SDN-DHA have not yet been determined. In addition, although several SDNs have been identified in the central nervous system of many vertebrate species, the homology and diversity of the SDNs are largely unknown.

First, as one step to clarify the physiological functions of the SDN-DHA, I performed a behavioral and histological analysis to determine whether the SDN-DHA of female mice is involved in the regulation of maternal behavior. Primiparous mothers and virgin female mice were subject to a maternal behavior test. Some of primiparous mothers and virgin females were not subject to the maternal behavior test. Animals were sacrificed after the behavior test was completed or at a concurrent time without exposure to pups. Brain sections from animals were double-immunostained for c-Fos, a neuronal activity maker, and calbindin, which is a maker of the BNSTp and CALB-SDN and is also useful to identify the SDN-DHA and measure the number of neurons expressing c-Fos in the SDN-DHA. As a result, virgin females that had lower performance in maternal behavior had many neurons expressing c-Fos in the SDN-DHA, and the cell number was increased by stimuli from

pups. The number of neurons expressing c-Fos in the SDN-DHA of primiparous mothers that exhibited higher performance in maternal behavior was lower than in virgin females and did not change with exposure to pups. These results suggest that a physiological role of the SDN-DHA is to suppress maternal behavior.

Although the SDN-DHA show a female-biased sex difference in ERα expression, there is little information about the molecular expression in the SDN-DHA and its sex differences. I performed a gene expression analysis of the SDN-DHA to find the genes, the expression of which differ between sexes. The SDN-DHA was isolated from Nissl-stained brain sections of gonadectomized male and female mice by using laser capture microdissection and subject to a DNA microarrary analysis. There were a variety of genes that are transcribed into coding RNA or non-coding RNA with sex differences. Of the genes showing higher expression in female mice, *snord116* and *oxt* were highly expressed in the SDN-DHA. In the result of qPCR analysis, the mRNA levels of *snord116* and *oxt* in the SDN-DHA were significantly higher in female mice than in male mice. Snord116 is a non-cording RNA. *Oxt* is the gene coding oxytocin, a neuropeptide neurotransmitter and hormone acting to modulate maternal behavior, suggesting that the SDN-DHA is involved in the regulation of maternal behavior.

In the last part of this study, I examined the brain of musk shrews (*Suncus murinus*) to determine the difference in the BNSTp between musk shrews and mice. In mice, the BNSTp is a SDN that is larger and contains more calbindin neurons in males than females. On the other hand, the BNSTp of musk shrews dose not express calbindin. In this study, I performed morphometrical analysis of the BNSTp in musk shrews using Nissl-stained brain sections. As a result, I found that the volume and neuron number of the BNSTp in male musk shrews were significantly larger than those of the BNSTp in female musk shrews. These results indicate that the BNSTp of musk shrews is a male-biased SDN as well as the BNSTp of mice, but there may a species difference in calbindin expression between musk shrews and mice.

In conclusion, this study suggests that the SDN-DHA of mice is involved in the regulation of maternal behavior. In addition, the molecular basis of the sex difference in the SDN-DHA of mice was profiled. Moreover, the BNSTp of musk shrews was identified as a male-biased SDN. These findings would be beneficial to better understanding of the sex difference in the brain and its diversity and homology.