Form 2

Dissertation Abstract

Report no.	(Course-based) No.1001	Name	CHAW KYI THA THU
Dissertation title	A Novel Sexually Dimorphic Area of the Hypothalamus: Morphological Characteristics and Mechanisms for Establishing Sex Differences(視床下部における新規の性的二型領域に関する研究)		

Abstract

Sex differences in the brain underlie sex- or gender-biased functions. Some neurological diseases, such as autism spectrum disorder, language impairment, attention-deficit hyperactivity disorder, cognitive impairment, and Alzheimer's disease, differ between sexes with respect to disease prevalence and symptoms. Understanding the sexual differentiation of the brain is required to better comprehend sex- or gender-biased brain functions and the etiology of sex-biased neurological diseases. The sexually differentiated brain includes sexually dimorphic nuclei (SDNs) that exhibit morphological differences between sexes or genders. The first SDN was discovered in the rat hypothalamus and termed the sexually dimorphic nucleus of the preoptic area (SDN-POA). The SDN-POA of rats is a male-biased SDN that is larger and has more neurons in males. Homologs of the SDN-POA have been identified in other species, including humans. Furthermore, the hypothalamus contains several SDNs that contribute to sex-biased physiological functions. However, there is still the potential to uncover novel SDNs and their physiological roles.

It has long been considered that testicular testosterone secreted during development is critical for sexual differentiation of the brain. In rodents, estradiol, which is locally synthesized from testicular testosterone by aromatase in the brain during the perinatal period, has masculinizing and defeminizing effects on the brain. Such actions of testicular testosterone were believed to be an exclusive factor in SDN formation, because estradiol or testosterone during the prenatal period acts to organize male-type SDNs, whereas female-type SDNs are formed independently of sex steroids. Recent evidence indicates that sex steroids affect the brain during puberty, which significantly contribute to sexual differentiation of the brain. Thus, gonadal hormones in the perinatal and pubertal periods play an important role in SDN formation.

Almost all brain regions including SDNs express estrogen receptor-α (ERα), a

nuclear receptor subtype for estrogens, and a sex difference in the expression of ERα was revealed in some SDNs. Formation of male-type SDNs in the mouse brain is disrupted by deletion of the ERα and aromatase genes, but not the gene of ERβ, another nuclear receptor for estrogens. Thus, neurons that express ERα and sense aromatized testosterone are key components of SDNs in the rodent brain. Recently, transgenic (tg) mice expressing green fluorescent protein (GFP) under the control of the ERα promoter (ERα-GFP tg mice) were generated. This characteristic of the tg mice may be useful for the analysis of the mechanisms responsible for SDN formation by monitoring ERα-expressing cells with GFP.

In this study, first, I examined the hypothalamus of adult ERα-GFP tg mice to determine whether sex differences in ERα-expressing neurons can be visualized by GFP. Fluorescence microscopy revealed the existence of many GFP-expressing cells in the medial preoptic area, medial preoptic nucleus, bed nucleus of the stria terminalis (BNST), striohypothalamic nucleus, and anterior hypothalamic area of adult ERα-GFP tg mice. In addition, female ERα-GFP tg mice had a greater number of GFP-expressing cells in a hypothalamic area sandwiched between two male-biased SDNs, which could be identified by the expression of calbindin protein and are termed the principal nucleus of the BNST (BNSTp) and calbindin-SDN (Calb-SDN). Thus, ERα-GFP tg mice are useful for the analysis of the sexual differentiation of the brain. The findings of this experiment using ERα-GFP tg mice suggested that the hypothalamic area sandwiched between the BNSTp and Calb-SDN (hereafter called the sandwiched area) is a potential female-biased SDN of the mouse brain.

Next, I examined the sandwiched area of wild-type mice to determine whether this area exhibits morphological sex differences. Brain sections obtained from wild-type littermates of ERα-GFP tg mice, and C57BL/6J and ICR mice were used for calbindin-immunohistochemistry and Nissl-staining to measure the number of neuronal and glial cells in the sandwiched area and for calbindin- and ERα-immunohistochemistry to measure the number of ERα-immunoreactive (ir) cells in the sandwiched area. As the results, the sandwiched area of female mice had a greater number of Nissl-stained neurons and ERα-ir cells, but not Nissl-stained glial cells, than male mice. Gonadectomy in adulthood did not affect such sex differences, indicating that the sandwiched area is sexually differentiated independently of gonadal hormones during adult period. In addition, I measured the length of the BNSTp, sandwiched area, and Calb-SDN. The length of the BNSTp and Calb-SDN in male mice was longer than

that in female mice. In contrast, the length of the sandwiched area was longer in female mice than in male mice. Interestingly, the total length of the BNSTp, sandwiched area, and Calb-SDN did not differ between sexes. These findings indicate that the sandwiched area of wild-type mice exhibits female-biased sex differences in the number of neuronal cells and the expression of ERα. There may be no strain difference in such sex differences of the sandwiched area.

Finally, to understand the mechanisms by which the morphological sex differences arise in the sandwiched area, I investigated the effects of gonadal sex steroids during the postnatal and pubertal periods on the formation of the sandwiched area. Wild-type male pups were subjected to orchidectomy on postnatal day (PD) 1 (day of birth). Wild-type female pups were treated with testosterone propionate (100 μg) or dihydrotestosterone (100 μg) on PD1, or estradiol benzoate (2 μg per day) on 5 consecutive days from PD1 to PD5. After maturation, they were histologically processed, and calbindin-immunohistochemistry and Nissl-staining were performed in the brain sections to measure the number of neurons in the sandwiched area. The data showed that the number of Nissl-stained neurons in the sandwiched area was increased in male mice by orchidectomy on PD1 and decreased in female mice by treatment with testosterone propionate, dihydrotestosterone, or estradiol benzoate. These findings suggest that the morphology of the sandwiched area is defeminized under the influence of testicular androgens during the postnatal period. Furthermore, I examined the effects of prepubertal gonadecotmy on the number of Nissl-stained neurons in the sandwiched area in adulthood. The number of Nissl-stained neurons in the sandwiched area of adult male mice was increased by orchiectomy on PD20. In contrast, the number of female mice was decreased by ovariectomy on PD20. Taken together, in the sandwiched area, testicular and ovarian hormones during puberty may act to decrease and increase neuron numbers, respectively, after the sandwiched area develops with or without the effect of testicular testosterone in the postnatal period.

In conclusion, I describe a novel sexually dimorphic area in the hypothalamus of mice. This currently defined sexually dimorphic area was sandwiched between the BNSTp and Calb-SDN that are known as male-biased SDNs. In contrast to the BNSTp and Calb-SDN, the sandwiched area exhibited female-biased sex differences. Female mice had many more neurons and higher expression of ERα in the sandwiched area than male mice. Hormonal manipulation during the postnatal and prepubertal periods reversed the sex differences of the sandwiched area in the direction opposite to the

BNSTp and Calb-SDN. The unique location and morphological characteristics of the sandwiched area indicate that male- and female-biased sexually dimorphic structures are organized in concert with each other so that the sex bias of the brain is balanced between sexes.