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Review Article

Brain Sexual Differentiation and Gender Development

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SUMMARY

Human behavioral sex differences are currently understood to result from a combination of social, cultural, cognitive, and biological mechanisms. To understand how gender identity as the sexuality of the mind is formed is important for understanding psychosexual problems of children and to consider how to manage patients with disorders of sex development (DSD), in which the development of gonads and genitals is atypical and it is difficult to determine the gender of boys and girls. There is consistent evidence that early testosterone exposure influences childhood gender role behavior, as well as gender identity and sexual orientation. In this review, we summarize the most relevant studies on the biological basis of sexual development. In particular, we focus on the impact of sex hormones and genetic background on development of sexual differentiation and gender identity, with introduction of our research using figure drawings by pediatric patients with congenital adrenal hyperplasia, which is also a DSD.

Key Words : Brain sexual differentiation ; Gender identity ; Androgen ; Congenital adrenal hyperplasia, Free drawing

INTRODUCTION

The process of sexual differentiation refers to the development of differences between males and females, which are widely observed in nature and also concern humans. One of the most sexually dimorphic human traits is gender identity, which is defined as the inner sense of self as a female, a male or an alternative gender that differs from male and female[1~5]. To clarify how gender identity related to sexuality of the mind, which is a manifestation of sexual differentiation in the brain, is formed is important to understand psychophysiological sexual problems of children and to manage patients with disorders of sex development (DSD), in which the development of gonads and genitals is atypical and it is difficult to determine the gender of men and women[6,7,8].

In this review, the most relevant studies on the biological basis of sexual development are summarized. In particular, the review focuses on the impact of sex hormones and genetic background on development of sexual differentiation and gender identity, with a discussion of the results of our related research on free drawing.

Psychosexual Differentiation and Gender Identity

Human sex can be divided into biological sex and psychic sex. The former includes genetic sex, gonadal sex, the appearance of the genitalia and somatic fea-

tures. In the latter, which indicates psychosexual differentiation, gender identity, gender role, sexual orientation and differences in cognitive ability are included. Gender identity, or the self-recognition of one's sex, refers to identifying oneself consistently as a male or female, and gender role indicates these sexually dimorphic behaviors. Sexual orientation is the name for the choice of a sexual partner, and there are three types : heterosexual, homosexual and bisexual. Sexually dimorphic cognitive abilities also occur, such as the greater ability of males than females in spatial cognition. Most biological sex is thought to be formed before birth, but the time of formation of psychic sex, and especially gender identity, is not clearly understood. However, it is generally considered that normally psychic sex develops in keeping with biological sex[1,3,9,10].

Sexual Differentiation of the Brain

In animals

In an early study, Phoenix et al.[11] found that treating pregnant guinea pigs with testosterone, a typical androgen, had enduring effects on the sex-related behavior of their female offspring. Since then, numerous studies of non-human mammals have documented the contribution of gonadal steroids, and particularly the testicular hormone, testosterone, to sexual differentiation of the brain and of behavior[3,13,14].

Sexual differentiation of the brain in rats can be recognized from their sexual behavior related to their reproduction activity. Sexual behavior such as mounting by male rats and lordosis in female rats are regulated by a nerve nucleus in the preoptic area (POA). This nerve nucleus in males is dense and bigger than that in females ; and since the nucleus enlargement is androgen dependent, it is referred to as the sexually dimorphic nucleus (SDN). The SDN-POA is the best characterized sex difference in the mammalian brain[3,13,14].

By administering testosterone, the SDN of rats is enlarged, and even a postnatal female rat behaves like a male rat. This androgen action is referred to as an organizational effect on the brain, and it is exerted during a circumscribed period of prenatal or early postnatal development ; the so-called critical period. Conversely, after a neonate male is castrated, it begins to act like a typical female, despite the fact that the rat is genetically male. Therefore, sexually dimorphic structures in the hypothalamus caused by endogenous or exogenous androgen action are considered to form the somatic basis of sex-specific behavior and sexual orientation. This also applies to primates, but it is generally considered that the influence of androgen tapers off in higher mammals[12,13,14]. Nevertheless, this view of androgen action on the brain needs to be modified, insofar as the administration of estrogen has been shown to masculinize the sexual behavior of animals, while administration of synthetic antiestrogens and aromatase inhibitors attenuate the organizing actions of endogenous or exogenous androgens[15].

In humans

To what degree the above responses are true in humans is still not clear, but sexual dimorphism of the hypothalamus and other regions of the brain in humans has been shown[16]. Testosterone secreted from fetal testes is elevated from about weeks 8 to 24 of gestation, with a peak at 8-14 weeks, during the period in which sexually undifferentiated internal and external genitalia differentiate into male or female. The increased testosterone may also simultaneously be associated with sexual differentiation of the brain[1~4], but in humans it is suspected that the effects on the brain occur later than differentiation of the internal and external genitalia[17,18] (Fig. 1).

The evidence that prenatal hormones affect development of gender identity is stronger, but far from proven. One indication that exposure to prenatal testosterone has permanent effects on gender identity came from an unfortunate case reported by Diamond and Sigmundson[19]. As an infant, the patient underwent a faulty circumcision and was surgically reassigned, given hormone treatments and raised as a girl. He was never happy living as a girl and, years later, when he found out what happened to him, he transitioned to living as a man. On the other hand, XY individuals born with an androgen receptor mutation causing complete androgen insensitivity are phenotypically female, identify as female and are most often androphilic, indicating that androgens act directly on the brain without the need for aromatization to estra-

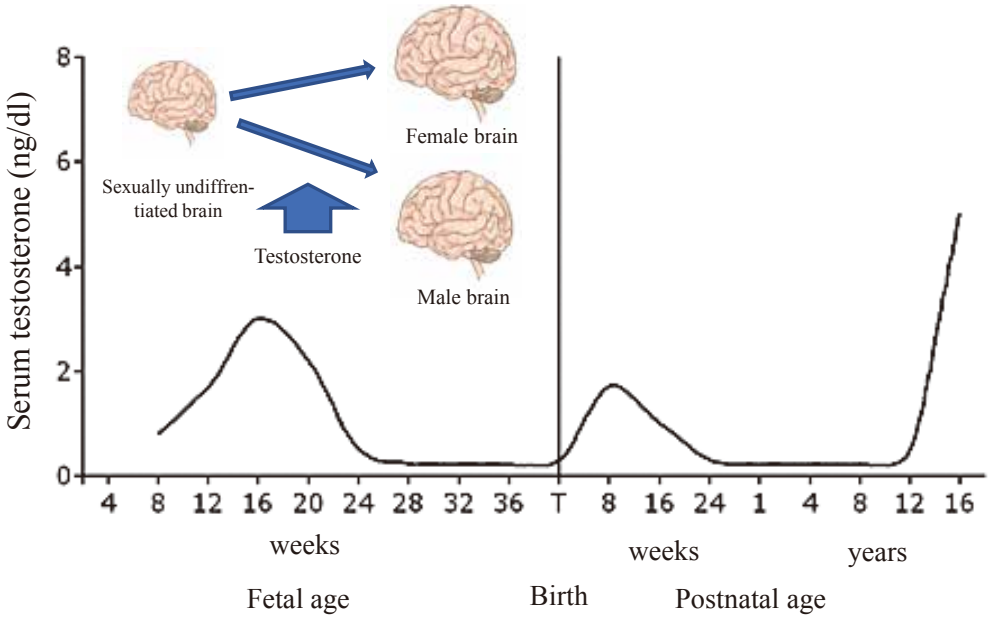

Fig. 1

Prenatal and postnatal changes of serum testosterone in humans. The relationship between fetal testosterone action and sexual differentiation of the human brain is shown schematically.

diol[20]. With regard to the role of estrogen in brain sexual differentiation, sexual dimorphism of the brain is thought to occur as a result of testosterone aromatization to estradiol in the brain of animals, but the precise role of estrogens in human psychosexual behavior remains unclear[1].

Postnatal Testosterone Surge and Masculinizing Effects on the Brain

Masculinizing effects of prenatal testosterone on human neurobehavioral development are well established (as explained in the next section). Also, an early surge of testosterone during months 1–5 postnatally, which is termed mini-puberty in male infants, is well documented and known to influence physiological development, including penile growth[21] (Fig. 1). However, the neurobehavioral effects of testosterone exposure during mini-puberty are largely unknown[3,12,22].

Under pathological conditions that impair testosterone secretion, such as bilateral congenital anorchia, functional secretory testes are present until 14–16 weeks of gestation before vanishing. This is an example of an individual lacking postnatal testosterone, and if the postnatal testosterone surge is critical for male-type sexual behavior, differences should be seen in this group of patients in comparison with men with functioning testes. However, no significant differences in general health, psychosocial and psychosexual functioning of patients with anorchia and healthy young adults have been found. Therefore, a postnatal testosterone surge does not seem to be critical for psychosexual development in men[23~25].

The possibility that testosterone during the early postnatal period may contribute to later autistic traits has also been discussed, but recent studies have shown that prenatal, rather than postnatal, organizational effects of androgen hormones influence the development of autistic traits in later life[26,27].

Gender Role Behavior in Girls with Congenital Adrenal Hyperplasia

The most common DSD is female patients with congenital adrenal hyperplasia (CAH). Much of the evidence regarding androgen effects on sexual differentiation of behavior comes from studies of individuals with intersex conditions, especially girls and women with CAH due to 21-hydroxylase deficiency.

CAH occurs in approximately 1 in 14,000 to 1 in 18,000 births and affects both male and females. It is most commonly caused by a mutation of the gene encoding the 21-hydroxylase enzyme, and it results in reduced cortisol production and elevation of cortisol

Fig. 2
Characteristics of free drawings by girls with CAH. Girls without CAH tend to draw the earth, flowers, and the sun, whereas boys draw images such as cars and trains. Generally girls do not show fighting in their pictures, but a fighting scene is drawn in the upper left of the upper picture. These images are thought to be due to the effects of androgens on the brain during the fetal period.

precursors. Because the cortisol pathway is not functioning properly, these precursors are shunted into the intact adrenal androgen pathway and some of these adrenal androgens are converted to testosterone. Consequently, females with CAH are exposed to high levels of androgens, including testosterone, beginning prenatally. This androgen elevation virilizes the external genitalia of females with CAH to varying degrees, and the brain of the female fetus is also exposed to androgen during the fetal period. Such elevated prenatal androgen exposure may have organizational influences on the brain and on later behavior and psychology[28,29,30].

If sexual differentiation of the human brain is affected by androgens present during sensitive periods of development, then females with CAH should show more "male-typical" and less "female-typical" behavior than control females. In fact, girls who received prenatal exposure to androgens have more interest in outdoor play and competitive sports and are more "tomboyish" than unaffected girls. It is characteristic of girls with CAH to play with boys' toys in childhood and this interest in boys' activities continues in adolescence[30,31].

In contrast, gender identity in females with CAH is female-typical. Most girls and women with CAH identify happily as females, although they seem to have somewhat reduced female identification. Thus, gender identity seems to be unrelated to the appearance of the genitalia (degree of virilization). This means that the few girls who have low identification as females are not necessarily those with the most virilized genitalia, and that normal appearing genitalia do not seem to be necessary for female-typical gender identity. Thus, gender identity is not simply related to the degree of prenatal androgen exposure or to genital appearance, and androgens appear to have a much smaller effect on gender identity than on gender role (toy play and activity interests)[32,33]. Ongoing studies are now focused on questions regarding the extent, nature, and mechanisms of hormonal influences on human behavior.

Effect of Prenatal Androgen on Free Drawing

Our group was the first to investigate the effect of androgen on free drawings by children. Analyses of drawings by girls with CAH and unaffected boys and girls were performed using masculine and feminine indexes. Sex differences in these indexes were clear in drawings by unaffected boys and girls, since these drawings did not or mostly did not contain characteristics typical of the opposite sex. Compared with pictures drawn by unaffected girls, those drawn by girls with CAH more strongly showed masculine characteristics. For these pictures, the feminine index was significantly lower and the masculine index was significantly higher compared to those for pictures drawn by unaffected girls. Furthermore, the masculine index for pictures drawn by girls with CAH did not differ significantly from that for pictures drawn by unaffected boys[3,34,35].

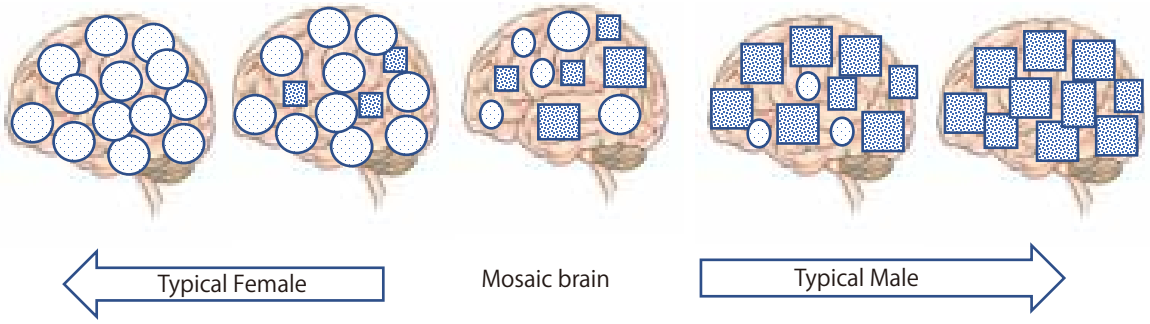

Fig. 3

Is the state of sexual differentiation in the brain mosaic? The brain cannot be dichotomized between men and women, and the gender difference in brain function seems to be mosaic. Brain function is thought to be partially shifted to either male or female.

▨ Portion differentiated into male.

◯ Portion differentiated into female

(This figure is a modified version of an original figure in reference no. 39 by the authors)

A picture drawn by one of the girls with CAH is shown in Figure 2 (lower right). At a glance, the picture is of objects such as stars and flowers, which are very colorful, and the yellow, pink and red are impressive. However, there are also objects such as a bus and wireless instruments that a girl without CAH would very rarely paint. These results suggest that androgen exposure during fetal life may contribute to shaping masculine characteristics in free drawings by children. Thus, mental functions such as the manner in which objects are perceived or expressed may be affected by prenatal androgen.

Current Understanding of Sexual Differentiation of the Human Brain

There are currently no findings of unique genes involved in sexual differentiation in the human brain[36]. As noted above, the male-typical or less female-typical behavior of XX patients with CAH is related to prenatal virilization of the brain. According to some studies, a higher proportion of young women with virilizing CAH, rate themselves as bisexual or homosexual. Although stronger credence is now given to the role of early hormonal effects on sexually dimorphic behavior in humans, prenatal virilization of the brain does not seem to be decisive in the development of gender identity[1,5]. A recent study of the sexual behavior of men with estrogen resistance or aromatase deficiency demonstrated that estrogens do not affect gender identity or sexual orientation in humans[37,38]. This may suggest that androgen is the major determinant of male gender identity in humans. Although a considerable part of sexual differentiation of the brain can be explained by the sex steroid hormone theory, research on a new theory of sex-specific transcriptional control mechanisms in the embryonic brain has been in progress for some time, and both hormone-dependent and hormone-independent mechanisms seem to be responsible for brain sexual differentiation.

Is Brain a Sexually Differentiated Mosaic?

Recently, there has been increasing attention on the idea that many normal physiological and behavioral processes are sexually dimorphic. The brain is undifferentiated very early in gestation, but in females every cell is XX and in males every cell is XY, and this can begin to have an impact on sex differences prior to the late gestational surge in androgen in males. These observations compel acknowledgement that there is no uniform "male brain" or "female brain," but that instead each male and female has a unique constellation of degrees of maleness or femaleness for each brain region or circuit, resulting in a brain mosaic (Fig. 3). Differences in the brain are broadly distributed, being found in regions relevant to reproduction, cognition, pain, anxiety, stress, and social behavior ; and hormonal, genetic, epigenetic, and environmental factors contribute differently in different regions. An understanding of the epigenetic mecha-

nisms through which an environment interacts with inherent biological signals is now emerging, and this may facilitate greater appreciation of the complex biological-environmental interactions that give rise to sex differences in the brain[39,40].

Conclusions

In this review, we examined how gender identity is formed as the sex of the mind, which is a manifestation of sexual differentiation in the brain. Prenatal androgens play an important role in sexual differentiation of the brain, and androgens lead to structural and functional gender differences in the human brain, but are not definitive. There are still many issues to be resolved in this field, including the question of whether the brain can be divided into male and female parts.

Conflict of interests

The authors have no conflicts of interest to declare.

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