Chapter

Regulation of Morphological and Functional Aspects of Sexual Dimorphism in the Brain

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Abstract

Sexual dimorphism of the adult brain regulates sex-dependent functions including reproductive and neuroendocrine activities in rodents. It is determined by sex steroid hormones during a critical perinatal period in female and male rodents. Sex steroids act on each nuclear receptor in the brain and control different physiological and neuroendocrine functions and behaviors. Several regions of the brain show evident morphological sex differences that are involved in their physiological functions. This review addresses and focuses largely on the role of sex-dependent differences in the brain, and their crucial functions in animal models. Particularly, recent intriguing data concerning the diversity of neuronal functions and sexual dimorphism are discussed.

Keywords: Sexual dimorphism, Sex steroid: Estrogen, ERα, ERβ, Neuronal plasticity

1. Introduction

Sexual dimorphism is characterized by morphological and physiological changes driven by sex steroids. In the rodent brain, it occurs during a critical period characterized by higher plasticity of neurons allowing changes in neuronal circuits and connectivity. For instance, hormonal manipulation during this time window, such as castration in males or replacement therapy in females (injections of androgen or estrogen), resulted in the conversion of intrinsic features and alteration of structures and functions of neural circuits in the brain. In rodents, critical time span for brain sex differentiation extends from embryonic day (ED) 18 to the postnatal day 10 [1], while in human it is exclusively embryonic day (ED12–22). Post this critical period, neuronal plasticity is lost and the effects of sex steroids can be diverted to activational effects in the brain. The mechanisms involved in defining the timing and duration of the neonatal critical period for the brain sexual differentiation remains to be determined. It is proposed that epigenetic modifications such as DNA methylation and histone acetylation might control the expression of genes implicated in brain sexual dimorphism [2].

The neuroendocrine systems, which control the action of sex steroids, including that on neural circuits, are differentiated in a sex-dependent manner, resulting in the regulation of reproductive and sex-specific behaviors. The actions of sex steroids in masculinization and feminization of the brain are mediated by steroid hormone receptors. In both human and nonhuman primates, young male and females show sex differences in toy preferences [3, 4]. Girls with congenital adrenal hyperplasia (CAH)

show to preference toward toys of males and to have decreased female-typical behavior [5]. These results argue that behavioral sex differences are caused by sex steroids. Estrogen is produced locally in the brain from testosterone by the aromatase cytochrome P450 enzyme [6, 7] and affects sexual differentiation by biding to estrogen receptor (ER) in rodents. Maternal and fetal estrogen can be bound by the α-fetoprotein produced by fetal liver cells and yolk-sac cells, thereby preventing their passage through the blood–brain barrier [8]. This mechanism results in female brain being free from estrogen. In contrast, in males, testosterone crosses the blood–brain barrier and is converted by aromatase to elicit sexual differentiation of the brain [6, 7, 9]. The effects of testosterone and its enzymatic derivative, estradiol, on their receptors are therefore critical for the sexual differentiation of the brain.

2. Region-specific regulation of the ER

2.1 Sex-specific differences in the anteroventral periventricular nucleus

The crucial role of estrogens in the sexual differentiation of the brain is mediated by estrogen receptors subtypes ERα [10, 11] and ERβ [12]. The amount of steroid hormone receptors differentiated and available development differs between sexes. The anteroventral periventricular nucleus (AVPV) is greater in size and cell number in females than in males [13, 14]. In the AVPV, the distribution of ERα is similar in both sexes, but its expression levels are higher in females than in males in prepubertal and adult rats [15]. In contrast, the distribution pattern for ERβ detected by nonisotopic *in situ* hybridization and immunohistochemistry is different between sexes [16]. Specifically, in females, a vast majority of ERβ-positive cells is located in the most medial portion of the AVPV, whereas the ERβ-containing cells in males are dispersed more laterally in the AVPV (**Figure 1**). The distribution of ERβ is reversed by neonatal hormonal manipulations [16]. Therefore, sex-specific physiological functions are predictable for sexual dimorphism in the AVPV.

2.2 ERβ sexual dimorphism in the AVPV

Steroid-mediated organization of the brain might involve cell apoptosis, cell migration, neurogenesis, cell differentiation and synaptogenesis. Estrogen and androgen induce programmed cell death [17] by the sequential activation of cysteine-dependent asparate-specific proteases (caspase) during the development of the hypothalamus [18] in the dimorphism of dopaminergic neurons in the AVPV [19–21]. The total number of ERβ-positive cells within the AVPV is not different between intact females and males [15, 22]. This is assumed to be caused by mechanisms other than apoptosis namely the sexual dimorphic expression of ERβ in the AVPV. The sexual dimorphic features of the brain caused by sex steroids do not always coincide with larger nuclei exclusively in one sex. Indeed, a region-specific ERβ gene expression is observed in the AVPV [22]. Moreover, the steroids might act on specific regions in the brain [22]. In brain slices from developing mouse brain, estradiol but not dihydrotestosterone induces and modulates neuronal migration [23, 24]. These results suggest that sexual dimorphism of ERβ in the AVPV might contribute to migration rather than apoptosis or neurogenesis.

2.3 Functional implications in ERα and ERβ localization in the AVPV

In the AVPV of female rats, a majority of ERβ-positive cells also express ERα [16]. It has been shown that ERα together with kisspeptin regulates ovulation, while ERβ is

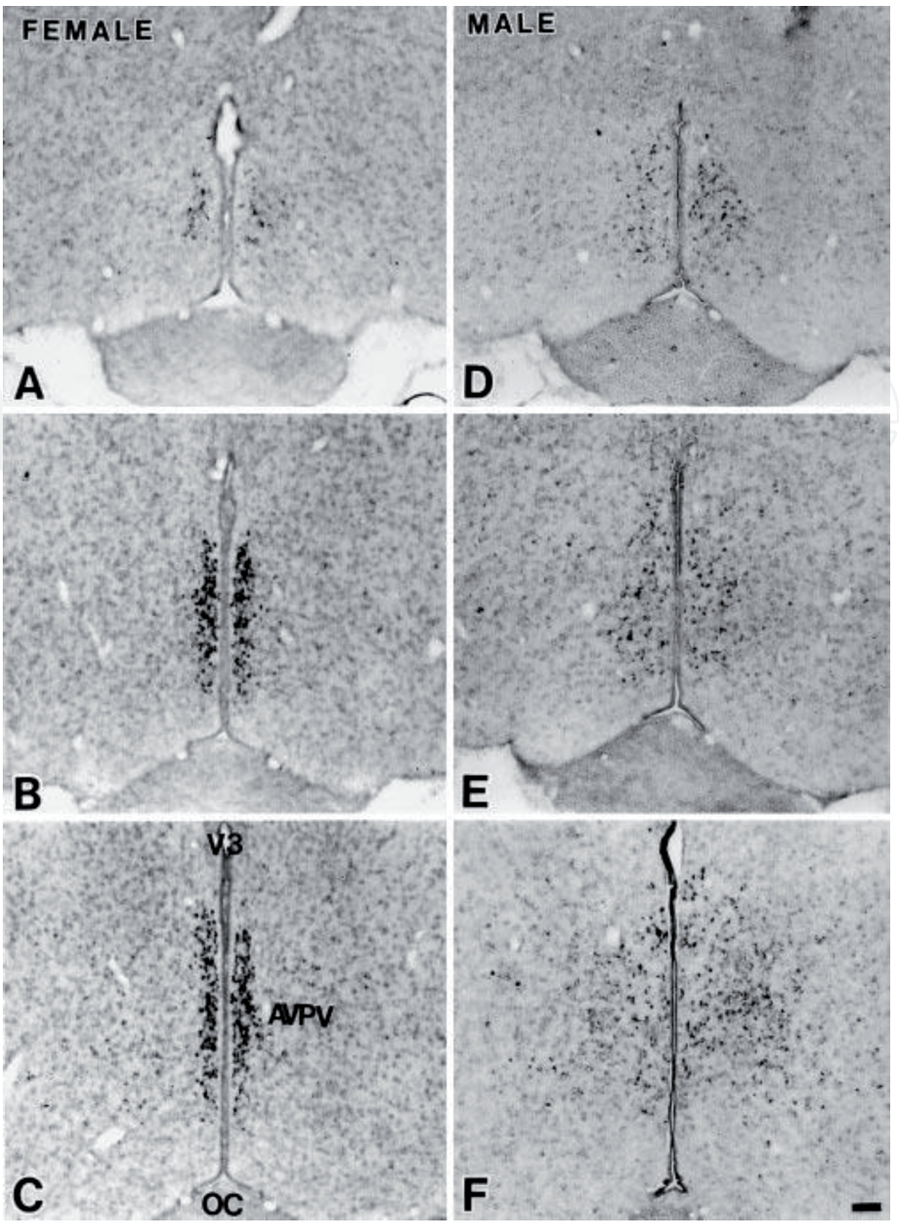

Figure 1.
Sexual dimorphism in the AVPV. ERβ positive cells aggregated densely in females (A-C), whereas the ERβ-containing cells in males (D-F) dispersed more laterally in the AVPV in the AVPV. Scale, 100 μm. From [16].

rather modified by these events [25]. At the molecular level, ERs bind to an estrogen responsive element (ERE) [26] after heterodimer formation [27], which allows the integration and collaboration of various signaling pathways for the completion of ovulation. The experimental infusion of antisense oligonucleotides in females results in decreased ERβ expression in the AVPV and consequently a persistent estrous [16]. Moreover, ERβ-positive cells and dopaminergic neurons have comparable distribution patterns in the AVPV [16]. Both ERα and ERβ have a role in the sexual dimorphism of dopaminergic neurons in the AVPV in both sexes [16, 28]. The secretion of luteinizing hormone (LH) is controlled by dopaminergic projections to neurons producing the gonadotropin-releasing hormone (GnRH) [29]. The cycle of female rats stalls ovulation state by small lesions of the AVPV [30]. Altogether, these data suggest that ERα and ERβ are colocalized with GnRH and are involved in LH secretion [31, 32]. In particular, ERα exerts a positive role for GnRH neurons, while ERβ exerts a negative control of those neurons [25]. Nonclassical ERE-independent ERα effects are involved in negative regulation on pulsatile GnRH secretion, while ERβ effects are involved in positive regulation on that secretion [31, 33]. It is still controversial to

regulate GnRH neurons by ERs. Considering the inherent male distribution pattern of ERβ, a peculiar characteristic of the dopaminergic innervation in the AVPV [28] might be responsible for the GnRH secretion in the brain of males.

2.4 Formation of the sexually dimorphic nucleus in the preoptic area

The sexually dimorphic nucleus in the preoptic area (SDN-POA) was first characterized by Nissl staining, revealing in a larger volume in the brain of male rats than that in the brain of female rats [1, 34]. The volume of this nucleus is altered by gonadal steroids during the perinatal critical period [1]. Somatostatin might also be involved in sexual dimorphism in the SDN-POA. Indeed, during development, cells positive for somatostatin are expressed in a sex-dependent manner in the SDN-POA. Sex reversal of the dimorphism of somatostatin expression is observed in orchidectomized males and estrogen treated female pups [35]. The somatostatin mRNA-positive cells are significantly more in males than in females, but eventually the difference recedes. Somatostatin expression in females is steady during the postnatal development. The transcription of somatostatin is transient and seems to contribute to the development of the SDN-POA. Somatostatin might prompt neuronal differentiation and survival via the somatostatin receptor.

Immunostaining against calbindin D28k, a major cytoplasmic calcium-binding and buffering protein, has been successfully used to identify the rat hypothalamus [36], SDN-POA [35, 37] and provides an alternative to Nissl staining [37]. Distribution of calbindin-labeled cells in the SDN-POA is similar to somatostatin in both sexes. It has been suggested that apoptosis has a role in sexual differentiation of the SDN-POA [38]. However, no difference in the total numbers of calbindin positive cells was observed in the SDN-POA after perinatal administration of bromodeoxyuridine in both sexes [39]. On the contrary, in the postnatal SDN-POA, these neurons still show an aggregated distribution in females, while they are dispersed laterally in males [39]. Altogether, these data suggest that, besides apoptosis, cell proliferation and migration might contribute to the morphological difference in the rat SDN-POA. Moreover, ERα are reported to be expressed in the SDN-POA [40], suggesting the presence of estrogenic action in the SDN-POA sexual dimorphism.

Moreover, Nissl stained SDN-POA had not been reported in mouse until recently identified by calbindin immunohistochemistry [41] (**Figure 2**). The morphological sex-dependent differences of the mouse SDN-POA were first demonstrated and established in terms of morphology and linked to gonadal steroid hormones during the prenatal critical period. Male mice have a greater number of calbindin-positive cells than females [41]. Similar differences within medial POA/anterior hypothalamic area (AHA) are observed in sheep, which are smaller in females than in males [42]. The volume of this nucleus in males is smaller in male-oriented than in female-oriented individuals. In humans, interstitial nuclei of the anterior hypothalamus (INAH) are considered comparable to those of rodent and sheep. The INAH is smaller in females than in males and smaller in homosexual men than in heterosexual men [43]. These results suggested that the sexual dimorphic nucleus in the two species is involved in sexual orientation. The male mice copulatory behavior and the preference for females is attributed to this difference in the SDN-POA [44–47]. Further functional analysis is required to completely understand the mechanisms involved in the sexual dimorphism of the SDN-POA.

2.5 Sexual dimorphic expression and function of ERs in the preoptic area

In the preoptic area (POA), ERα expression is much higher in females than in males [48]. This sex difference occurs during the perinatal period. After birth, the

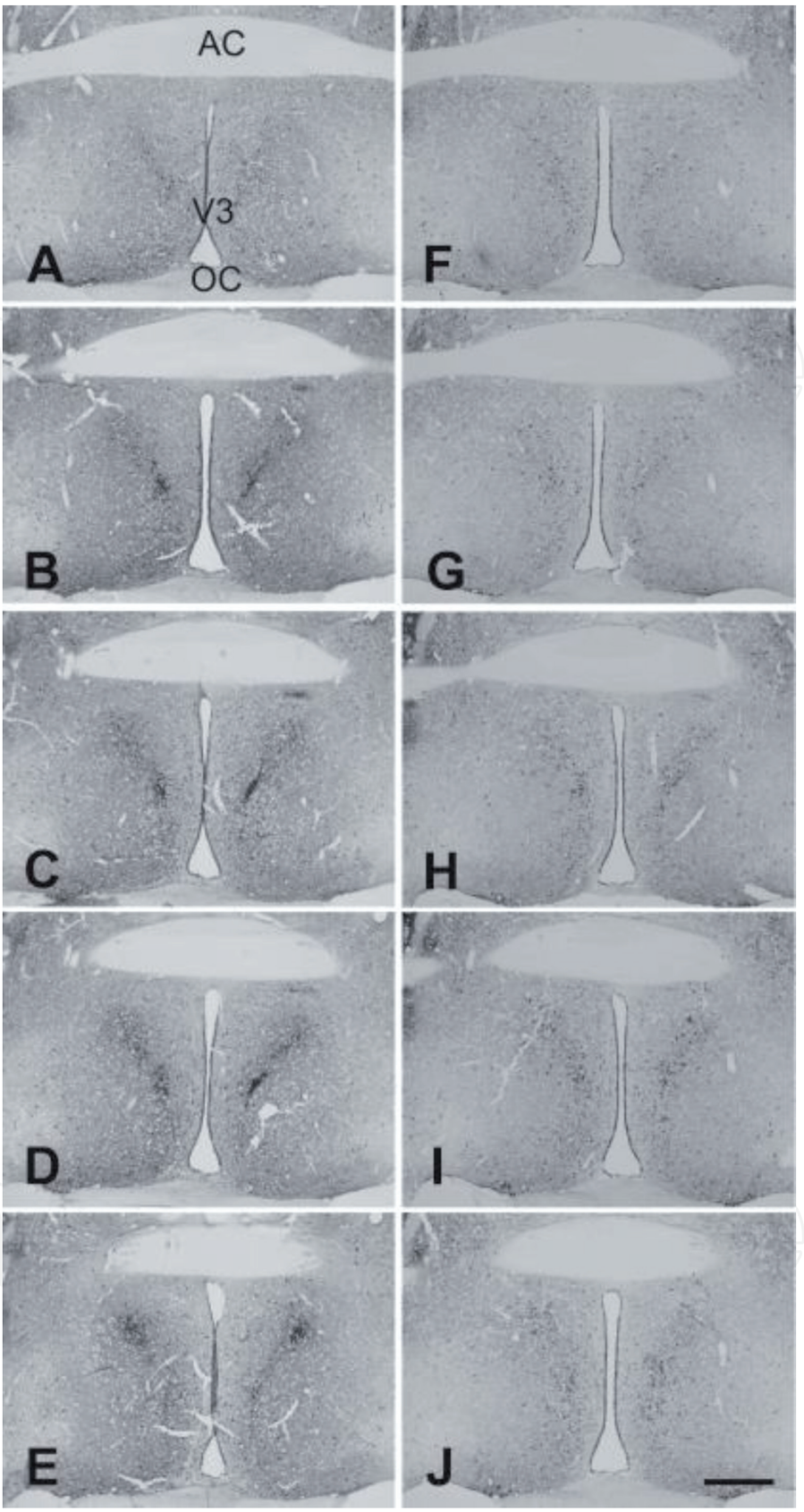

Figure 2.
Sexual dimorphism in the SDN-POA. Calbindin (CB)-immunoreactive cells in the mouse SDN-POA in males (A-E) and in females (F-J) in the rostral-caudal direction. In males (B-D), but not females, a cell aggregate of CB-positive cells is prominent. Sale, 400 μm. From [41].

expression of ERs is down regulated in the POA by estrogen [49]. The decreased ERα expression occurs in both sexes but the differential expression in the POA between females and males persists throughout life. Although the ERα levels

are higher in females than in males, a comparable distribution pattern of ERα is observed [16, 48]. The POA has been implicated to be involved in steroid activation of the male copulatory behavior [50]. In particular, dopamine neurons in the mPOA prompt male sexual behavior [51]. ERα and oxytocin containing neurons in the mPOA participate to control copulatory behavior in male rats [52–54]. In females, the POA and the adjacent bed nucleus of the stria terminalis (BNST) is considered essential for controlling maternal [55–58] and mating behaviors [59]. ERα in the mPOA is involved in the regulation of maternal care, maternal aggression and sexual behavior [56]. ERβ is detected by *in situ* hybridization and immunohistochemistry in the medial preoptic nucleus (mPOA) and more caudally in the BNST [16] (**Figure 3**). In males, ERβ in the mPOA is involved in aggressive behavior [60]. Overall in rodents, identical brain regions control specific behaviors depending on the sex. Recently, it is shown that the male-typical mounting behavior and female-typical pup retrieval behavior are induced by ERα located in the same region of the POA [61]. These data suggest that the sex specific neural circuits are able to control opposite behaviors. Therefore, sex-typical behaviors are likely induced by the harmonic expression of sex specific receptors together with sex steroid. Besides the neural circuit with a high degree of plasticity in the sexual dimorphic nervous system assuring precise sex-specific behavior events, there may be a possible the involvement of circumstances in ensuring responsiveness of the sex steroids.

2.6 Functional diversity of the ventromedial hypothalamus

The volume of the ventromedial hypothalamus (VMH) is larger in males than in females [62, 63]. ERα and ERβ are expressed in the VMH of rodents [22, 48].

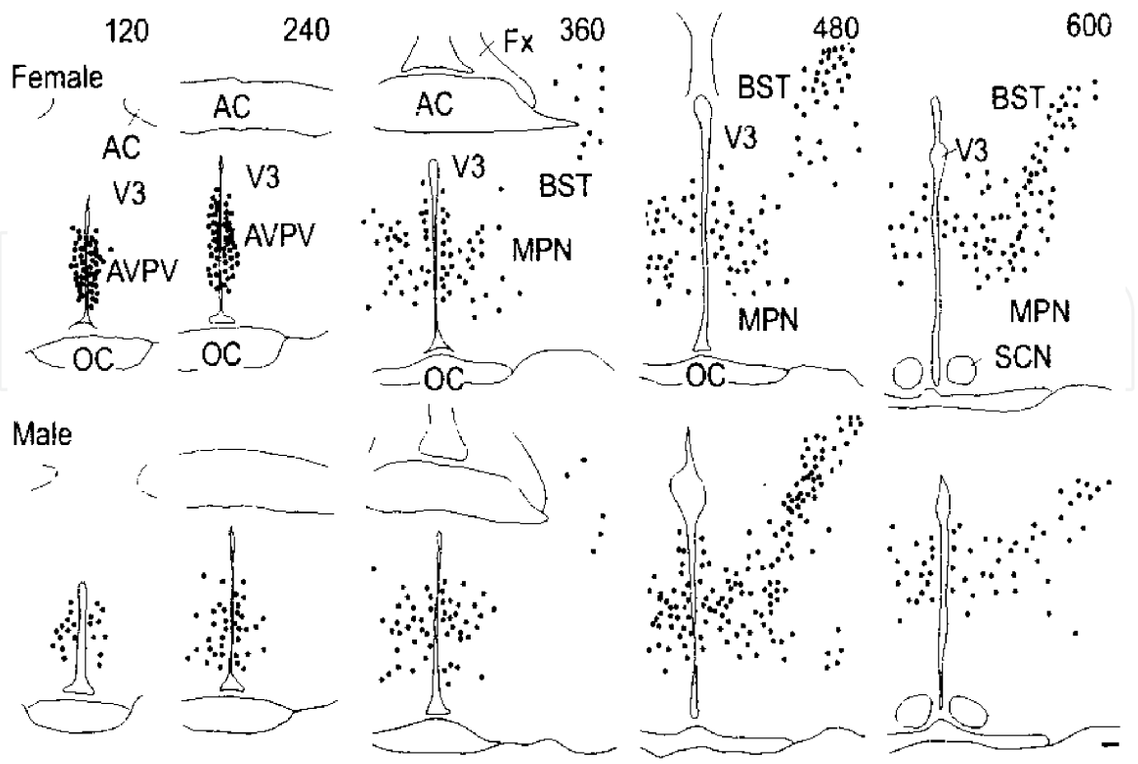

Figure 3.
Schematic representation of the distribution of ERβ mRNA-positive cells in the forebrain of rats through rostrocaudal axis. Scale, 100 μm. AC, anterior commissure; AVPV, anteroventral periventricular nucleus; BST, bed nucleus of the stria terminals; Fx, fornix; MPN, medial preoptic nucleus; OC, optic chiasm; SCN, suprachiasmatic nucleus; V3, third ventricle. From [16].

However, ERα expression is abundant in the ventrolateral portion of the VMH and is higher in female rats than in male rats [48]. The sex difference is most likely due to the conversion of testosterone into estrogen, which downregulates ERα expression. Moreover, the aromatase signal in males is more robust than in females [64]. A sex difference in ERβ expression is observed in both postnatal day 14 and in the adult rat brain, indicating that the sexual dimorphism is also maintained throughout life [22]. ERβ expression in the adult VMH is downregulated by estrogen or testosterone administration. The difference in expression is reversed by administration of estrogen in female rats or orchidectomy male rats. This sexual dimorphism is entirely attributable to the effects of sex steroids on the brain organization and plasticity during the critical neonatal period of the brain. Estrogen, converted from circulating androgen in males, downregulates ERα and ERβ expression in the VMH [22, 48] and consequently physiological functions. Estrogen together with progesterone in the VMH induces female sexual reproductive behavior such as lordosis, sexual receptivity and odor preference [65].

In adult males, the expression of ERα is lower than that in females. Cells in the male VMH are activated during fighting [66]. In these processes, ERα is involved in sexual [67] and aggressive behaviors in mice [68, 69], whereas ERβ is assume to be inhibitory to the aggressive behavior [68]. Other studies have demonstrated that male sexual behavior is not affected by ERβ in the VMH [70], but is profoundly regulated by ERα and the androgen receptor (AR), suggesting a possible distinct role for ERβ and ERα on each behavior. Opposing social behaviors, such as mounting and attack, are regulated by ERα [67] or progesterone receptor [71] cells located in discrete regions of the VMH. Sex steroid receptor expression in the VMH is induced by environmental hormonal milieu during the critical period and in turn controls the dynamic action of the sex hormones on sex-specific behavior in adults [66, 72]. These data suggest that males and females seem to exhibit identical neural circuits in the VMH, but the activated receptors might contribute to inducing the sex-typical behavior. The sex-specific neural circuit dictated by sex steroids could work in conjunction with estrogen-mediated ERs.

3. Alternative mechanism for sexual dimorphism in the medial amygdala

The medial amygdala (MeA) is larger volume in males than in females [73] and this difference is abolished after castration in males and androgen treatment in females [74]. In adults, the size and volume of neurons is modified by circulating androgen. After castration of adult male rats, the cell soma size in the posterodorsal MeA (MePD) is similar to the one observed in females [74]. However, the number of MeA neurons in both sexes is not affected by adult androgens [75]. Steroid hormones also influence the organization of the MePD during the neonatal period [76, 77] and its metabolite, estrogen, results in masculinization of the MePD. ERα and ERβ are abundantly expressed in the MePD [22, 48, 78, 79] where the aromatase enzyme is also detected [80, 81]. ERs mediate estrogen-induced modifications in the MePD associated with masculinization and male-specific behaviors [82]. However, the masculinization in the MePD in the adult brain is mostly driven by circulating androgen [82]. Both the action of ER [82] and AR [83] in the MePD on the size of neuronal somas and in the sexual behavior mostly occurs in the adults [82]. In adults, there is no sex-dependent difference in ER subtype and expression in the MePD, but there is a sexual dimorphic expression of ERβ but not ERα in newborns [84]. Neonatal hormonal manipulations could not reverse the sex differences in ERβ in both sexes, suggesting that ERβ-mediated estrogen actions are not involved in the sexual dimorphism in the MePD. Furthermore, ERβ is highly expressed in the

MePD of adult female and male rats and is not affected by gonadectomy or estrogen treatment in both sexes [22]. Therefore, the ERβ expression also acts independent of activity in this structure.

In the MePD, sexual dimorphism involves mechanisms distinct from other regions of the brain. The MePD receives inputs from the olfactory and pheromonal systems, suggesting a functional role of this structure in sex arousal and regulation of adult social behaviors, including mating, aggressive [85, 86], and territorial behavior [87]. Acquisition of mating stimuli induces Fos in the ERs in the MeA [88]. Finally, the mechanisms induced by the ERs in the MeA and those involved in sexual stimuli [89], gonadotropin secretion [90], ovulation [91] sex and courtship behaviors [87], onset of puberty [92], parenting, and reproduction [85, 89, 93] still remain to be identified.

4. Conclusion

Sexual dimorphism is characterized by morphological differences in several regions of the brain. Morphological sex differences in the POA/AHA and the INAH were revealed in sheep and human brains, which are assumed to be important for determining sexual orientation. Expression of the phenotypes i.e., behavioral sex differences, are suggested to be derived from morphological sex differences in the brain. However, the morphological sex differences are subtly evident in other human brain regions; hence, their association with functional sex differences in the human brain remains controversial. Consequently, CAH results in masculinized female brain, thereby leading to male-typical preferences, which are the congenital characteristics inherently caused by steroid and not acquired by learning. Striking sex differences in animal models contribute in establishing the mechanisms of sexual dimorphism in the brain of all living beings.

ER expression levels contribute substantially to the physiological and behavioral differences. However, the extent to which the amounts of ER control the development of sexual dimorphism remains to be clarified. Sex-specific neural circuits activated by sex steroids might contribute to the functional role of ERs activated by estrogens. Recently it was evidenced that a high neuronal plasticity rate in neural circuits is necessary to ensure precise sex-specific responsiveness to sex steroids. The mechanism involved in the regulating the local action of sex steroids remains to be elucidated. Particularly, the expression and regulation of genes implicated in sexual dimorphism must be investigated.

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Declarations interest

None.

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