Dimorphism of Human Brain: The Basis of the Gender Differences

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For mammals, sexual differentiation starts at conception when a fetus inherits a couple of heterologous (XY) or homologous (XX) sex chromosomes. Until the sixth week of gestation embryonal gonadal development is bi-potential, than, according to genetic sex, embryonal gonads differentiate in testes or ovaries. In presence of Y chromosome, fetus will develop testes; hormonal products of the testes, mainly testosterone, then induce the male phenotype by early permanent programming or organizational effects and later transient acute or activational effects, which disappear after withdrawal of the hormones. In the absence of Y chromosome, the fetus develops ovaries, and in the absence of male-like levels of testosterone, the female phenotype emerges. The activating effects of ovarian hormones together with endocrine and exogenous influences enhance female characteristics at puberty and beyond determining phenotyping sex [1].

Several studies affirm that gonadal determination may be controlled by various genes, such as gene Sex-determining Region Y (SRY) on short arm of Y chromosome (Yp11) involved in gonadal differentiation to testes. SRY codes for a protein containing sequence High Motility Group box (HMG) which is transient expressed in cells planned to become Sertoli cells. Moreover, gonadal determination seems to be influenced by genes, in particular, Wilm's tumor-related gene-1 (WT-1) and steroidogenic factor-1 (SF-1). Mutations of these genes in both male and female sexes are involved in the pathogenesis of agenesis and dysgenesis gonadal syndrome. It is evident that primordial gonads are inclined to an intrinsic development to female gonads and only the presence of genetic factors on Y chromosome or activated by developing testes are able to masculinize. Currently only one gene coded on X chromosome (Xp21.3), dosage-sensitive sex reversal-adrenal hypoplasia congenital on

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the X chromosome (DAX-1) seems to prevent possible gonadal evolution to testes in female mammals [2].

Sex chromosomes play a direct role in establishing sex differences throughout the body including the brain, thus some differences depend on chromosomal constitution and are gonadal independent. Nonetheless, increasing evidences suggest that for the large majority of neural sex differences, the modifications in gonadal hormones seem to play a dominant role. Sex differences in the central nervous system (CNS), probably may develop to direct actions of gonadal steroids which can determine neural tissue differentiation, moreover, sexual differentiation of CNS occur later than gonadal differentiation thus both processes can be independently influenced [3].

Steroid hormones synthesized by the gonads and adrenal glands easily cross the blood–brain and the blood–nerve barriers and rapidly accumulate within the nervous tissues, except for their conjugated forms such as the steroid sulfates, which cannot easily enter the brain. Neurons and glial cells possess enzymes necessary for progesterone, testosterone, dehydroepiandrosterone (DHEA), and estradiol metabolism (aromatase, 5-alpha reductase (5a-R), mainly in neurons, 3-alpha-hydroxysteroid dehydrogenase (3a-HSD), mainly in type 1 astrocytes). The activities of these steroid-metabolizing enzymes are strongly influenced by the differentiation process of the precursor stem cells into terminally differentiated CNS cells. Neurons and glial cells coordinately metabolize steroid hormones, thus forming a functional unit; as both the endocrine glands and the local metabolism contribute to the pool of steroids present in the nervous tissues and the sex and age-dependent changes in circulating levels of steroid hormones may reflect changes in brain levels. While steroid-metabolizing enzymes induce the CNS to be able to modify circulating steroids, the CNS is also able to synthesize steroids from cholesterol, at least in part, independently of peripheral steroidogenic glands secretion leading to the production of a series of potent steroidal compounds. These brain-produced steroids have been named “neurosteroids”, and have been found to exert important regulatory actions on neurons and glial cells [4] (Fig. 1.1).

The so called “neurosteroids” influence the neurobiology of sexual function acting by genomic or non-genomic effects. Genomic actions of neurosteroids are carried out directly interacting with their receptors at nuclear membrane level or indirectly throughout their effects on neuropeptides (oxytocin, beta-endorphin, etc.), neurotransmitters (dopamine, serotonin), and neurosteroids metabolites (mainly allopregnanolone). Non-genomic actions are mediated through integrate or associated membrane receptors and the activation of intracellular cascades of events determining rapid neuronal and pituitary activation via biochemical pathways of AMPc and MAP-kinases; thus resulting in a modulation of Ca2+ channels and exerting neuroprotective effects in contrast to neurotoxins and oxidative stress [5].

Estrogens have long been known to play a crucial role on coordinating many neuroendocrine events that control sexual development, sexual behavior, and reproduction. 17-β-estradiol is the primary biologically active form of estrogen in mammals which is critical for sexual differentiation of the brain, indeed it organizes neural circuits and regulates apoptosis of neurons leading to long-term differences

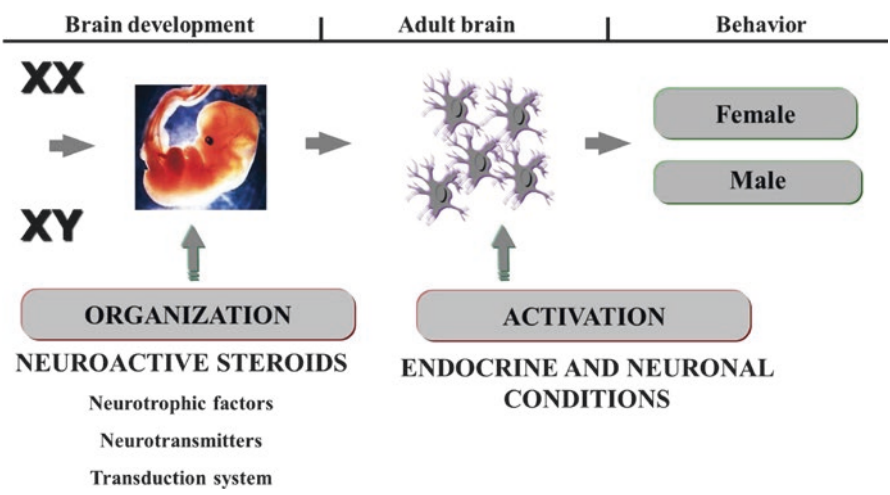

Fig. 1.1 Brain sexual differentiation is a multisignaling process presenting sex steroids as key modulators in different steps

in the male and the female brain. In addition to its role in development, estradiol prevents neuronal cell death in a variety of brain injury models, modulates learning and memory, and promotes the formation of synapses as well as cellular apoptosis. The physiological effects resulting from estradiol actions in target tissues are mediated primarily by two intracellular receptors ERα and ERβ. Both estrogen receptors together with progesterone receptors A and B (PR-A, PR-B) and androgens receptors (ARs) are observed in neurons and glia in the brain and are expressed throughout the brain with distinct patterns in different brain regions and with different levels of expression in males and females during development and in adulthood. Consequently, sexual dimorphism of human brain seems to be characterized by functional and structural differences. Functional differences are determined by hormonal and enzymatic actions or pathway modulating masculinization or feminization of different regions of CNS, while structural differences are determined by different distribution of ERs, ARs, PRs, enzymatic isoforms, and neuronal population in different cerebral areas [6].

Relating to functional differences, as previously mentioned, 17-β-estradiol is a crucial biologically active form of steroid in mammals involved in sexual differentiation of the brain, nonetheless, several experimental and pre-clinical data suggested that testosterone (T), acting on the brain, seems to regulate reproductive function, sexuality, and emotional behaviors in both sexes in a different gender-related fashion. In addition, T exerts analgesic and anxiolytic properties, affects mood and cognition, and promotes synaptic plasticity in the rat model. T also prevents neuronal death in different experimental models of neurodegeneration, and decreased T levels in plasma may represent a risk factor for the development of neurodegenerative diseases in humans. T brain effects may be directed or modulated by its metabolites, therefore, T can be aromatized to estrogen or metabolized

to dihydrotestosterone (DHT) by 5a-reductase (5a-R), and DHT can be further reduced by 3-hydroxysteroid dehydrogenase (3-HSD) to 3-androstanediol (3-diol), a neurosteroid Gamma-Aminobutyric Acid-Aergic (GABA-A) agonist with anxiolytic properties. These two enzymatic pathways, aromatase and 5a-R-3HSD, are widely distributed in CNS, affecting reproductive (i.e., hypothalamus) and non-reproductive function (i.e., hippocampus, cortex) of gonadal steroids [7].

Interestingly, DHEA and its sulfate metabolite DHEA-S may act on CNS differentiation directly, by modulating several activities in different neuronal populations or as substrate for the conversion in T and DHT in such CNS target regions of androgens and estrogens. In this view, it is remarkable to highlight that brain DHEA and DHEA-S concentrations are 5–6 times higher than peripheral concentrations and several pre-clinical studies demonstrated the presence of steroidal precursors such as cholesterol and lipid derivates in mammalian brain. The effects of DHEA and DHEA-S on CNS are mediated by direct interaction with GABA-A receptors, thus blocking Cl^- channels in a dose-dependent manner and resulting in increased neuronal excitability. Experimental data also suggested putative effects of DHEA on N-methyl-D-aspartate (NMDA) and sigma (λ) receptors. DHEA administration to gonadectomized rats increased concentrations of neurosteroids not related to DHEA metabolism such as allopregnanolone (3-hydroxy-5-pregnan-20-one) (AP), in the hippocampus, in the hypothalamus, in pituitary, and in peripheral circulation and improved mnemonic ability, thus suggesting neurotrophic effects on neurons and glia cells. Moreover, gonadectomy reduced synaptic density on dendritic spines and CA1 pyramidal neurons in both male and female rats while T and DHT administrations are able to reestablish that. In this view, it has been hypothesized that the preservation of physiologic synaptic density may be an androgen-dependent process which can be elicited with different gender-specific mechanisms since that in male rats, contrary to female rats, it is not necessary synthesis of intermediate estrogens. Moreover, the ability to restore hippocampal synaptic density is not directly related to androgenic potency since that it has been demonstrated that DHEA and DHT stimulation activity on dendritic density are similar [8].

Estrogens can also increase the activity of the enzymatic pathway (5a-R)—3-hydroxysteroid-oxidoreductase, which converts progesterone into 5-dihydroprogesterone and AP, respectively. Progesterone and synthetic progestins can affect brain and peripheral content of AP divergently, both in humans and in experimental animals, suggesting distinct hormonal effects on the enzymatic pathways involved in the synthesis and release of these neurosteroids [9]. AP is a neurosteroid produced by the central nervous system, adrenals, and ovaries. AP is a 3-, 5- reduced metabolite of progesterone by the complex 5a-R-3HSD. It is a potent endogenous steroid that rapidly affects the excitability of neurons and glia cells through direct modulation of the GABA-A receptors activity. AP exerts neuropharmacological properties with hypnotic/ sedative, anxiolytic, anesthetic, analgesic, and anticonvulsive function [10]. In addition, AP exhibit neurotrophic/neuroprotective actions, reducing cell death, gliosis, and functional deficits after traumatic brain injury in rats and in experimental models of Alzheimer's disease, enhancing myelination/remyelination process. Interestingly, several experimental studies suggest that

AP positively affects all aspects of sociosexual activities, enhancing exploratory, antianxiety, social, and sexual function [11].

Genazzani and collaborators, in experimental work on male and female gonadectomized rats model, studied the effects of the administration of subcutaneous T at the dose of 10–100 μg/kg/day for female rats, and 1–5 mg/kg/day for male rats, or DHT at the doses of 1–10 and 100 μg/kg/day for females, and 0, 1–1 and 5 mg/kg/day for males, or E2V (0.05 mg/Kg/day). Ovariectomy (OVX) and orchidectomy (OCX) induced a significant decrease in AP in frontal and parietal lobe, hippocampus, hypothalamus, anterior pituitary, as well as in serum. In OVX rats, T replacement, as well as E2V, significantly increased AP content in all brain areas and in peripheral circulation, whereas in OCX, T and E2V did not actively result in influencing AP concentration in frontal and parietal lobe, while it produced a significant rise in AP levels in the hippocampus, hypothalamus, anterior pituitary, and serum. Conversely, DHT replacement had no effect on AP levels anywhere or at any administered dose, either in males or in female rats. The author concluded that gender difference and T therapy may affect brain AP synthesis/release during the reproductive aging. This effect becomes particularly evident in the brain of OVX animals, where the content of this specific neurosteroid is much more responsive than male animals to testosterone replacement. Moreover, it has been suggested that T administration should be, at least in part, dependent on a gender difference in the aromatase activity; therefore, a sexually dimorphic activity of aromatase is widely described during the fetal and postnatal life, and also, the expression and the activity of this enzyme were dimorphically affected by gonadectomy and by T replacement, supporting the hypothesis of differential enzymatic regulation also for neurosteroidogenesis [12].

The same group, focusing the attention on the homeostasis of CNS and the role of neurosteroids in the hormonal setting, investigated the gender response of endogenous opioid system to hormonal changes. The endogenous opioid system modulates responses to stress, learning and memory acquisition; it is involved in emotional regulation, pain mechanisms, and the reward system, and it is altered in various pathological states. β-endorphin (β-END) is the endogenous opiate that has received the most attention. It has been speculated that β-END may play a key role in the mechanism of sexual arousal and pleasure in both sexes and its effects seems to be inversely dose-related therefore, the administration of low physiological dose of opiate have facilitative effects and high dose exhibit inhibitory effects. Similarly, the administration of naloxone at low doses to women was able to enhance pleasure during orgasm while higher doses show contrary effects, reducing sexual arousal and orgasmic pleasure. In addition, the administration of exogenous opiates can induce an intense feeling of pleasure which has been associated to orgasm, followed by a state of relaxation and calm. Gonadal steroids are increasingly recognized as crucial factors modulating the endogenous opioid system in both sexes, suggesting the presence of additional hormone-related, neurobiological mechanisms for gender difference in brain function. The administration of above-mentioned doses of T, DHT, and E2V to male and female gonadectomized rats showed relevant results. T administration to OVX rats exerted a powerful impact on the endogenous endorphin system; therefore, it enhanced β-END concentration not only at hypothalamic level but also in several hypothalamic

structures, affecting the activity of endorphinergic neurons in the hippocampus as well as in the frontal and parietal lobes. In contrast, the endorphin content of these hypothalamic structures was not affected in male rats by orchidectomy or by any steroid replacement therapy; thus suggesting that the cerebral structures receiving the endorphinergic peptide exhibit a sex-based difference in opioid system sensitivity to gonadal hormones. Since the effect of estrogen treatment was the same for both sexes, the physiological basis for this sex difference in β-END sensitivity to T therapy might depend, at least in part, on a sex difference in aromatase activity; the authors concluded that sexually dimorphic aromatase activity characterized fetal and postnatal life and this study highlighted that the expression and the activity of the enzyme were dimorphically affected by castration and T replacement [13].

As previously mentioned, structural differences are determined by different distribution of ERs, ARs, PRs, enzymatic isoforms, and neuronal population in different cerebral areas. In particular, ERα and ERβ are expressed in the amygdala, the hippocampus, in different areas of cerebral cortex, in the cerebellum, and in the hypothalamus where estrogen may determine structural and behavioral sex-specific characteristics.

Two brain regions that show robust estradiol-induced organizational changes are the preoptic area (POA) and medial basal hypothalamus (MBH), both of which are critical for sexual behavior. Estradiol-induced organizational changes are perhaps best exemplified within the POA, an area containing the medial preoptic nucleus (MPN), the sexually dimorphic nucleus of the preoptic area (SDN-POA), and the anteroventral periventricular nucleus (AVPV). The MPN is critical to the control of male sexual behavior and the SDN-POA is a sub-region within this nucleus that has been implicated in partner preference [14]. The AVPV is important for gonadotropin secretion and is believed to be the source of control of the LH surge essential for ovulation in adult females [15]. In the perinatal male brain, estradiol derived from testosterone stimulates opposing events in the SDN and AVPV, protecting neurons within the SDN-POA from apoptosis by enhancing NMDA receptor expression, while provoking expression of proapoptotic proteins such as TRIP, Bad, and Bax to induce apoptosis in the AVPV [16, 17]. In addition to modulating cell death, estradiol also mediates sex differences in synaptic patterning in the MPN by inducing synthesis of the cyclooxygenase enzymes, COX-1 and COX-2, thereby increasing the production of prostaglandin-E2 [18]. Acting via the EP2 and EP4 receptors, PGE2 activates protein kinase A and allows for glutamate-induced activation of AMPA receptors and formation of dendritic spines, the postsynaptic contact points for excitatory synapses [19]. Ultimately, the increased production of PGE2 in the male brain results in a two to three times higher density of dendritic spine synapses compared to females, and interestingly, this higher spine density positively correlates with the degree of masculinization of sexual behavior [20]. Thus, three key cellular responses, cell survival, cell death, and synaptogenesis, are all mediated by estradiol within one brain region, the preoptic area. The divergent effects of estradiol are mediated via the estrogen receptor (ER), in particular the ERα isoform. In addition to exerting organizational effects on the physiology of the POA, estradiol also induces permanent changes in synaptic connectivity in the MBH. The ventromedial nucleus of the hypothalamus (VMN) is a key region for regulating female sexual behavior. Within the VMN, male neurons have

twice the number of dendritic spines and more dendritic branches than females as a result of neonatal hormone exposure. Estradiol produces sex differences in synaptic organization in this region by rapid activation of PI3-kinase which enhances glutamate release from presynaptic cells, thus provoking dendritic spine outgrowth from postsynaptic neurons. Here, too, the ERα isoform is the critical mediator of estradiol action, although the initiating steps in the signal transduction cascade appear to begin at the membrane via rapid activation of PI3 kinase, and more interestingly, the requirement for ER is restricted to the presynaptic membrane despite the induction of changes in neuronal morphology within the postsynaptic neuron. The enduring organizational changes produced by estradiol within the neonatal brain enable circulating gonadal hormones in the adult to activate sexually differentiated brain regions, such as the POA and the MBH, in a sex-specific manner. Thus, in adulthood, estrogens and progesterone act on a female brain to regulate pulsatile LH release and induce estrous cyclicity and female sexual receptivity, whereas testosterone reaches a masculinized adult brain to activate male sexual behavior [19].

As a concluding remark, some sex differences may cause differences in function, in other cases sex differences exist to ensure that function is similar in males and females. In other words, some sex differences compensate for physiological differences that if left unchecked may be maladaptive. Sex differences that perform a compensatory role may become evident when the system is perturbed. A specific example comes from looking at something as apparently basic as cell death programs in neurons. In response to hypoxia, or other conditions mimicking stroke, neurons die in both sexes of rats and mice [21].

A sex difference in a physiological process is one of nature's ways of demonstrating how that process can be modulated. Sex differences in the vulnerability to a disease may similarly reveal factors that are protective in one sex, thereby suggesting strategies to prevent or ameliorate that disease. This is especially true for many neurodevelopmental disorders, where "sex" explains more of the variance than any other known contributing factor. In humans, some treatments are known to be more effective in one sex than the other, and optimal drug doses for men and women may differ. We ignore these things at our peril and we believe that ignoring sex differences in the brain, however they arise, compromises best practices in biology and medicine, in some cases with substantiated negative health effects [22].

A deeper understanding of these mechanisms will ultimately lead to our understanding of the molecular mechanisms underlying differences in the male and female brain, and importantly, differences in how the male and female brain may be able to respond to neuronal insults encountered with injury, neurodegeneration, and normal aging.

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