Structural and molecular brain sexual differences: A tool to understand sex differences in health and disease

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Sex differences are present both in the genotype and in the phenotype of all vertebrates, and they have been evidenced also within the central and peripheral nervous system. Earlier studies on brain sex differences suggested a relatively simple view based on (1) the presence of sexually dimorphic circuits in the hypothalamus (or in regions related to reproductive behaviors), (2) the action of gonadal hormones to masculinize the brain, and (3) the gonadal steroids' action to modulate gene transcription through nuclear receptors. These assumptions are today contradicted by the findings accumulated in the last 20 years. We know now that mechanisms determining sexual dimorphisms may vary according to location and species, and may involve several factors, as genes, epigenetic factors, gonadal hormones and neurosteroids. Sex differences were also revealed by epidemiological studies in several neural pathologies. This suggests that the approach to understand the genesis of these pathologies, should involve specific attention to interactions among genes, gonadal and brain-born steroid hormones, epigenetic and environmental factors.

Key words: Medial preoptic region; Vasopressin; Vasotocin; Kisspeptin; Dopamine; Estradiol: Neuroactive steroids; Adult neurogenesis; Epigenetics

1. Introduction

Sex differences in the phenotype of living animals are very diffuse both in invertebrates and vertebrates. The reproductive organs are a typical example of such differences, they are differentiated for their morphology, for the production of gametes (different in male and female), and for their endocrine functions. Other, so-called, secondary sex characteristics are: the body size, ornamentation, fat tissue distribution, some parts of the skeleton (i.e. pelvis, skull), hair, and many other structures. Also several behaviors are sexually dimorphic and this implies the presence of sex differences in brain neuroanatomy and/or neurophysiology.

In birds and mammals, sex differences have been demonstrated at chromosomal level, with a couple of chromosomes (sex chromosomes or heterochromosomes) that are different among males and females. Particular genes on these chromosomes [the gene *DMRT1* on Z chromosome of birds (Smith et al., 2009) and the gene *SRY* on Y chromosome of mammals (Sinclair et al., 1990)] are responsible of the male sex determination. The primary goal of these genes is to induce the development of male gonads. The central and simpler hypothesis (that is now under criticism in view of recent discoveries, see Lenz et al., 2012) is that animals with *SRY* or *DMRT1* gene will develop testes whose hormones will induce the differentiation of male phenotypes, whereas in the absence of *SRY* (or with a diminution of *DMRT1*), the genetic program will induce an ovary whose hormones will determine the female phenotypes. Therefore, according to this dogma the phenotypic differences between male and females are based on more or less precocious exposure to the "right" hormone and this induce the expression of that characteristics for the rest of the life (organizational effects of steroid hormones).

Berthold (a German physiologist) was probably the first to observe, in 1849, a sexual difference in animal behavior and to link it to the differences in the gonads. For this reason he is considered the father of behavioral endocrinology (Beach, 1981; Jorgensin, 1971). In his experiment, Berthold noted that, in addition to phenotypical characteristics as the presence of a combe and of wattles, the roosters (male chickens) were more aggressive than females (hens) and they copulate with hens, whereas these last do not copulate with other hens. Castrated rooster did not develop comb and wattles, in addition, they were not aggressive and did not copulate. But, when castrated roosters received a transplanted testis, this became functional (producing sperm) and the morphological and behavioral phenotypes of intact roosters were restored. This experiment was performed a long time before some concepts as

hormones, neural basis of behavior, sex determination, and sex differences were clarified. However it clearly demonstrate that the products of the testes can stimulate the differentiation of male external morphology, of male typical behaviors and of specific neural circuits controlling these behaviors.

Only after more than 100 years, Phoenix and coworkers (1959) published a seminal paper demonstrating that the alteration of prenatal gonadal hormones environment may lead to adult alteration of sexual behavior, thus establishing the difference among "organizational" and "activational" effects of gonadal hormones. After this first study, several experiments have been performed to study every known sex difference in behavior. At the same time, many studies were dedicated to investigate the presence of sexually dimorphic circuits, nuclei or other structures potentially related to sexually dimorphic behaviors (Abel and Rissman, 2012; Arnold and Gorski, 1984; Arnold et al., 2003; Panzica et al., 1995).

The first significant evidence of an anatomical difference at the hypothalamic level (number of synapses on dendritic spines in the dorsal medial preoptic area, MPOA) was published by Raisman and Field (1971), that lately demonstrated that this difference is organizational (Raisman and Field, 1973). Due to the technical limitations of the studies at ultrastructural level these differences are difficult to find and it is not possible to apply this approach for the description of large brain structures. After these studies, Gorski and coworkers found a more easily detectable neural difference: the presence of a sexually dimorphic nucleus (SDN) within the MPOA whose volume and cell number is higher in male than in female rat (Gorski et al., 1978; Gorski et al., 1980). Sexually dimorphic structures, organized during embryonic or postnatal development were subsequently described in different vertebrate species (for reviews see Breedlove, 1992; Panzica et al., 1995; Panzica et al., 1996; Simerly, 2002), including humans (Swaab and Fliers, 1985).

In oscine birds, Nottebohm and Arnold (1976) found sexually dimorphic nuclei (larger in males than in females) in the telencephalic regions controlling the emission of song. This difference is triggered by testosterone (T) in the adult canaries (Nottebohm, 1980). These studies demonstrated for the first time a deep connection among seasonal changes in reproductive behavior and changes in the morphology of related circuits in intact birds (Nottebohm, 1981).

The picture resulting from these early studies was relatively simple (at least for rodents and canaries): (1) sexually dimorphic circuits are located in the hypothalamus or in other regions

controlling behaviors related to reproduction; (2) brain masculinization depends by the presence of gonadal hormones during specific (critical) periods, whereas their absence drives the brain to the female sex; (3) the steroids, solely produced by gonads, act through their nuclear receptor and directly modulate gene transcription.

All these three assumptions are today at least partly contradicted by the new findings accumulated in the last 20 years (Arnold, 2009b).

2. Sexually dimorphic circuits or nuclei in the central nervous system.

From the first ultrastructural and histological studies many other techniques were employed to detect sex differences in the central nervous system. The chemical neuroanatomical techniques (including immunohistochemistry, autoradiography and *in situ* hybridization) have detailed the presence of neurotransmitters, neuropeptides, enzymes involved in their synthesis, or receptors. In this way the number of end points to be considered to study the sex dimorphism increases a lot. In some cases, the neurochemical markers detailed structures already evidenced in histological studies. This is the case of the quail medial preoptic nucleus (POM) that was at first described with Nissl's staining (Viglietti-Panzica et al., 1986), and later its location, volume, and steroid-induced plasticity were confirmed by using immunohistochemistry for the enzyme aromatase (ARO) (Aste et al., 1994). A different example is the SDN-MPOA of the rat. It was the first sexually dimorphic nucleus that was observed in the mammalian hypothalamus with histological methods (Gorski et al., 1978), however, for a long time, researchers failed to find a murine counterpart of the SDN in Nissl-stained sections. At the beginning of this century a subdivision of the SDN was found to be positive for calbindin-D28k (whose function in this context remains unclear but probably it is related to cell survival and apoptosis) and this subdivision was sexually dimorphic and responsive to gonadal hormones treatments as the SDN (Sickel and McCarthy, 2000). Later, other researchers found that a similar cluster of calbindin-positive cells exists also in the preoptic region of the mouse. This marker delineates a sexually dimorphic region that cannot be evidenced with Nissl's staining (Edelmann et al., 2007) and, as the rat SDN, is dependent by gonadal hormones to sexually differentiate (Budefeld et al., 2008).

Immunohistochemical and *in situ* hybridization studies have been largely used to investigate sex differences of neuropeptidergic circuits. Among several systems that have been described,

two were particularly detailed in different vertebrate species. The first one is the rat parvocellular sexually dimorphic arginine-vasopressin (AVP) system, located outside the hypothalamus in the bed nucleus of the stria terminalis (BST) and in the medial amygdala. Its projections reach several extra-hypothalamic locations, in particular the lateral septum, the ventral pallidum, the hippocampus and various brain stem nuclei (De Vries et al., 1985; Gu et al., 2003). Cell bodies and projections are strongly sexually dimorphic, having males more cells and higher density of positive fibers than females (De Vries et al., 1985). Similar sexually dimorphic cell groups were observed in different mammalian as well as non-mammalian species (in this case the peptide is the arginine-vasotocin, AVT). The mechanisms determining the sex differences may vary (see below), but the endpoint (the dimorphism) is similar in the different models (for a review see De Vries and Panzica, 2006). The parvocellular AVP/AVT system shows gonadal hormones receptors and is activated by them, however its sexual dimorphism seems not to be related to the presence of estradiol (E_2) during the critical period (Pierman et al., 2008; Plumari et al., 2002), but probably to a direct genomic effect (De Vries et al., 2002) or to the presence of a functional androgen receptor (Allieri et al., 2013).

Another strongly sexually dimorphic peptidergic system that has been more recently investigated in several mammalian and non-mammalian species is the kisspeptin system. This system is characterized by two groups of neurons: the first one is located in the anterior preoptic area (AVPV) and the second one is in the arcuate nucleus (ARC). These neurons project mainly to the gonadotropin releasing hormone (GnRH) neurons that control the secretion of gonadotropins from the hypophysis (Dungan et al., 2006). The system is therefore extremely important for the control of reproduction. It is sexually dimorphic with a higher number of cells and fibers in the female than in male and is strongly gonadal hormones dependent in the adult (Kauffman et al., 2007). The kisspeptin system has been characterized in various vertebrate species discovering at least three types of molecules (Kiss1-2-3) and four types of receptors (KissR1-4) (for a review see Pasquier et al., 2014). In mammals Kiss1 has been linked to the development of puberty, and several investigations have elucidated the implicated mechanisms (Clarkson et al., 2010).

Sexually dimorphic pathways (characterized by their content in neuropeptides, neurotransmitters or enzymes) have been described in several regions of the CNS outside the hypothalamus and the limbic system. For example the enzymes tyrosine-hydroxylase (TH) and dopamine-β-hydroxylase (DBH) show a sex dimorphism in their expression or regulation within the rat locus coeruleus (the main nor-adrenergic nucleus in the brain, Luque et al.,

1992; Thanky et al., 2002). The expression of the enzyme for the GABA synthesis (GAD65) is sexually dimorphic in discrete regions of the hypothalamus, but also in extrahypothalamic regions as the amygdala or the hippocampus (Perrot-Sinal et al., 2001). Dorsal root ganglia and the dorsal horn of the spinal cord, show a sexually dimorphic expression of estrogen receptors (ERs) and this is probably one of the reason why female rat is more sensitive to deep pain than male (Papka and Mowa, 2003).

An interesting example of diffuse sex dimorphism is the expression of calbindin in several populations of neurons (outside the small group located in the preoptic region): i.e. the frontal cortex, thalamus, hippocampus, amygdala and cerebellum. In the frontal cortex and cerebellum of juvenile mice, calbindin expression is sexually dimorphic with females having about twice positive cells than males (that is the opposite of what observed in the hypothalamus). In addition, the mechanisms determining these sexual differences are also different for the two regions: in the frontal cortex the dimorphism depends by both ERα and sex chromosomes, but in the cerebellum sex chromosomes are the main (sole?) factor (Abel et al., 2011).

The dopaminergic system is largely diffused in the brain. The majority of dopaminergic cells have been observed in the substantia nigra (projecting to the forebrain: striatum, cortex and limbic system) or in the ventral tegmental area (projecting to the frontal cortex, the amygdala and the nucleus accumbens). The dopaminergic neurons are usually identified by the presence of the tyrosine hydroxylase (TH), the rate-limiting enzyme involved in dopamine synthesis. Sexually dimorphic expression of this enzyme has been observed both in the substantia nigra (Ma et al., 2007) and in the ventral tegmental area (Brown et al., 2015). Fibers containing TH have been described in many brain regions, where they have frequently a sexually dimorphic distribution (Kritzer and Creutz, 2008).

In the hypothalamus there are two populations of TH-positive elements that show a sexually dimorphic distribution. The first is located in the AVPV and the second in the ARC nucleus (Arbogast and Voog, 1990; Balan et al., 2000). Recent studies have shown that at least part of these two populations of dopaminergic neurons express also kisspeptin (Kauffman et al., 2007).

Many other neurotrasmitters, neuropeptides and/or their receptors are expressed in a sexually dimorphic way throughout the entire central nervous system. Sex differences have been detected also at molecular level in mechanisms that are potentially widespread in the

brain. For example electron microscopic studies discovered sexual differences in spine synapse density in females but not males in the caudal part of nucleus accumbens (Wissman et al., 2012). In the hippocampus, brain-born E_2 rapidly suppresses inhibitory synaptic transmission blocking GABA release from CB1 receptor-containing inhibitory presynaptic endings in females but not in males (Huang and Woolley, 2012). A recent study demonstrated that this suppression is due to inositol triphosphate (IP3) generation, activation of the IP3 receptor, and postsynaptic endocannabinoid release (Tabatadze et al., 2015). The complexes of ERα, glutamate receptor and IP3R are present in both sexes, but are regulated by E_2 only in females (Tabatadze et al., 2015).

The development of imaging techniques opened a new field of investigations for the human brain. In fact, it is now possible to select more homogeneous groups of volunteers, and it is easier to study also sex differences in the human brain. A recent study discovered unique sex differences in brain connectivity by using diffusion tensor imaging in more than 900 youths (aged 8-22 y, more than 400 males and 500 females). Males had greater intra-hemispheric connectivity, whereas in females more inter-hemispheric connectivity has been observed (Ingalhalikar et al., 2013). However, another study performed on more than 1,400 subjects revealed extensive overlap between the females and males characteristics for gray matter, white matter, and connections, revealing that most brains are "mosaics" of features, some more common in females, some more common in males, and some common in both sexes (Joel et al., 2015), but these conclusions and the applied statistical tools were critically questioned (Del Giudice et al., 2016). Also optogenetic techniques have been recently applied to investigate sex differences in the inferior temporal cortex of macaque monkeys performing a facial gender-discrimination task (Afraz et al., 2015).

These and many other data are therefore contrasting the hypothesis that sexually dimorphic circuits are located only in the hypothalamus or in other regions controlling behaviors related to reproduction: their distribution is much larger and involves several parts of the central nervous system. In addition, the endpoints that can be considered are numerous, starting from large anatomical differences (as the volume of the rat SDN or of the quail POM) and ending to very subtle neurochemical differences (as the modulation of GABA release).

3. Mechanisms determining the development of sexual differences within the central nervous system

There is a strong connection among gonadal hormones and the development of sex differences of the body including the brain, however the mechanisms may vary in different species and other factors have important, even preeminent, roles. Early in development, the increased volume of the SDN in rat males depends by the availability of E_2 locally produced from circulating T by the action of the enzyme ARO. In the female, blood circulating E_2 is blocked by the alpha-fetoprotein (Bakker et al., 2006), therefore the increase of SDN volume happens only in males. Estrogens may prevent cell death in the male SDN by inhibiting capsases (Choi et al., 2008) or/and by regulating anti-apoptotic proteins (Gill and Christakos, 1995). This mechanism is valid for the rat SDN (and may be for other mammals), but in other vertebrates, as galliform birds, E_2 is acting in an opposite way inducing demasculinization of the volume of the medial preoptic nucleus (Aste et al., 1991) and of the sexually dimorphic parvocellular AVT system in the BST (Panzica et al., 1998). The regulation of cell proliferation during the embryonic period (in particular around the E12-14, that is the end of the critical period in galliforms) seems to be also in this case one of the key mechanisms, however no clear hypothesis has been presented (Bardet et al., 2012).

Another example is the sexual differentiation of the AVPV (located in the most medial part of the MPOA). The AVPV is larger in volume and cell number in females as compared with males (Bleier et al., 1982; Simerly et al., 1985). This sex difference results by an androgen-dependent mechanism, in fact cell number is reduced in females pre- or post-natally treated with T propionate (Arai et al., 1994; Murakami and Arai, 1989). Androgens and androgen receptors are also important for the sexual differentiation of other brain circuits, as demonstrated by the alteration of nitrergic population in the MPOA, VMH and BST nuclei (Martini et al., 2008) or of the AVP system in the BST and medial amygdala (Allieri et al., 2013) in androgen receptor defective (Tfm) male rats.

Estrogens and androgens are produced by the gonads, that differentiate through a genetic mechanism linked to the presence of X and Y chromosomes, in particular, in mammals, by the presence of the *Sry* gene. It is therefore possible that some brain differences are directly depending by a chromosomal effect and not by an epigenetic effect of gonadal hormones. The TH system represents a very peculiar system to investigate this mechanism. In fact, in cell cultures extracted from the mesencephalic region at the embryonic day 14 (before the surge of T in the rat male embryo), the uptake of dopamine (a marker of the functional maturation of these neurons) as well as the number of cells were sexually differentiated (Beyer et al., 1992; Beyer et al., 1991; Engele et al., 1989; Reisert et al., 1990). This suggests that the genome

should be responsible of sex differences in rodent mesencephalic dopaminergic system (Reisert and Pilgrim, 1991). This hypothesis was confirmed by experiments demonstrating the hormonal independence of these sex differences in long-term steroid-exposed mice (Sibug et al., 1996).

An interesting genetic model to test the effects of the genome is the so-called "four core genotypes (FCG)" mouse model (Arnold, 2009a; Arnold and Chen, 2009) that comprises mice in which sex chromosomes (XX vs. XY) are unrelated to the animal's gonadal sex. The males are either XY or XX^{+Sry}, and the females are XX or XY^{-Sry}, all males have testis and females have ovaries. Thus, the FCG model may provide information regarding the non-gonadal origins of sex differences. For the mesencephalic dopaminergic system, cell cultures from the mesencephalon of E14 FCG mice confirmed the effect of genetic sex on these sex differences. Cell cultures from XY males or XY^{-Sry} females had more TH- cells than XX or XX^{+Sry} cultures (Carruth et al., 2002). Therefore, the genes located in the Y chromosome (and not the *Sry* which is determining the development of gonads) are important for the sexual differentiation of this circuit.

In contrast to these results, the sexual differentiation of the TH-containing neurons of the AVPV is strictly dependent by the perinatal hormonal environment. In fact, the disruption of the ERα feminizes the system, inducing a 3 times increase in the number of TH neurons of AVPV in ERαKO males in comparison to the wild type, while no changes have been detected within the AVPV of female ERαKO mice. The androgen receptor seems not to be involved, in fact Tfm mice contain the same number of TH-immunoreactive neurons in the AVPV (Simerly et al., 1997). The action of E_2 in males is probably linked to an increase of the apoptosis, as demonstrated *in vitro* and *in vivo* by using drugs inhibiting capsase that induced an increase of TH-immunoreactive neurons (Waters and Simerly, 2009).

Outside the hypothalamus, gonadal hormones have an important role as regulative factors of adult neurogenesis (Galea et al., 2013). In the hippocampus they differentially affect cell proliferation and survival in male and female rat: repeated administration of E_2, decreased the survival of new neurons, increased cell proliferation, and decreased cell death in the female, whereas they have no effect in male **(Barker and Galea, 2008)**. Cellular proliferation decreases in ovariectomized females, and it is restored by acute treatment with E_2 (Tanapat et al., 2005). In male rats, androgens (T and dihydrotestosterone), but not E_2, stimulate cell survival (Spritzer and Galea, 2007). In the subventricular zone (SVZ), the modulatory effects of

gonadal hormones are highly variable according to sex, species, treatment and strain. In a recent study (Farinetti et al., 2015) we demonstrated that E_2 and T, but not dihydrotestosterone, increase proliferation in the SVZ of adult castrated male rats, whereas ovariectomized female rats treated with E_2 or T do not show any significant effect.

Recently, major attention has been paid to the epigenetic mechanisms occurring at the DNA level. In a recent study (Nugent et al., 2015) the authors found that gonadal steroids act in the MPOA to reduce activity of DNA methyltransferase enzymes, decreasing DNA methylation and inducing the activation of masculinizing genes. Pharmacological inhibition of these enzymes induced the masculinization of neuronal markers and the appearance of male sexual behavior in female rats. Also conditional knockout mice for one of the isoforms of DNA methyltransferase enzymes (Dnmt3a) showed masculinized sexual behavior in female. These data show that "brain feminization is maintained by the active suppression of masculinization via DNA methylation" (Nugent et al., 2015).

In conclusion, the organization of sexually differentiated circuits is based not only on circulating gonadal hormones secreted by gonads, but also by locally synthetized E_2, by androgens, by genes located in the sex chromosomes, not limited to the Sry, and by epigenetic factors.

4. Sex difference in neuroactive steroids.

Data so far discussed seems to suggest that only sex steroids produced by peripheral glands (or their direct metabolites, as the case of E_2 derived from T) are able to influence sexual differences in the nervous structures. However, the last three decades of investigations have clearly indicated that steroids affecting nervous function are not only those coming from the periphery (i.e., steroid hormones) but also those directly synthesized in the nervous system (i.e., neurosteroids). These two classes of steroid molecules are now integrated in the term *neuroactive steroids* (Giatti et al., 2015; Melcangi et al., 2008; Melcangi and Panzica, 2006; Rupprecht and Holsboer, 1999). Indeed, nervous system expresses molecules involved in the transport of cholesterol into the mitochondria, such as steroidogenic acute regulatory protein (StAR) and translocator protein, as well as several steroidogenic enzymes (Melcangi et al., 2008). Thus, nervous system possesses the capability to synthesize and to metabolize neuroactive steroids. Importantly, StAR, the cytochrome P450 side chain cleavage (i.e., the first

steroidogenic enzyme) and ARO show sex differences in the nervous system (Lavaque et al., 2006; Lavranos et al., 2006). Interestingly, it has been recently demonstrated that, in the FCG mouse model, sex chromosome complement determines sex differences in ARO expression in brain structures. Thus, the bed nucleus of the stria terminalis and the anterior amygdala of 16 days old XY and XY^{-Sry} mouse embryos show higher aromatase expression than the brain of XX and XX^{+Sry} embryos (Cisternas et al., 2015). In physiological conditions the levels of several neuroactive steroids show sex differences within the nervous system (Melcangi et al., 2016),

The so-called classical action of steroid hormones is performed through nuclear receptors that, after binding the appropriate ligand, show a conformational change allowing receptors to bind directly to specific steroid response elements in the promoter regions of specific genes (O'Malley and Means, 1974). However, neuroactive steroids, including E_2, (Balthazart and Ball, 2006) may induce rapid changes that occur within seconds to milliseconds, this is called the non-classical action and it is due to G protein–coupled and ligand-gated ion channel membrane receptors (for a review see King, 2008). Many receptors are involved, as glycine receptors, metabotropic sigma type 1 receptors, N-methyl-D-aspartic acid (NMDA) receptors and γ-aminobutyric acid type A (GABAA) receptors. Recently, a G-protein coupled estrogen-sensitive receptor (termed G-protein estrogen receptor 1, GPER1) has been described within the brain (Hazell et al., 2009). Several studies elucidated the distribution and subcellular localization of this receptor in the brain, as well as some of the roles that this receptor may play in the brain particularly in the control of synaptogenesis and spinogenesis and the impact that estrogens may have over brain diseases (for reviews see Hara et al., 2015; Sellers et al., 2015; Srivastava and Evans, 2013). In agreement with these concepts, several observations have been obtained indicating that neuroactive steroids play important roles in sexual function and behavior (see for a review King, 2008).

In conclusion, the three main assumptions reported in the introductions, are contradicted, at least partly, by the recent findings. In particular: (1) sexually dimorphic circuits are located all over in the brain and the spinal cord. (2) The organization of sexually differentiated circuits depends by several mechanisms including circulating gonadal hormones, locally synthetized E_2, androgen and estrogen receptors, genes located in the sex chromosomes, and epigenetic factors. (3) The action of steroids in the central nervous system includes both classical and non-classical mechanisms. Finally, brain-born steroids (i.e., neurosteroids) are involved in many physiological regulations.

5. Sex differences in neurodegenerative and psychiatric disorders

Sex differences are not only present in physiological status but also in neural pathologies. Indeed, as reported in this special issue and by an extensive recent literature (Young and Pfaff, 2014) sex differences have been observed in psychiatric disorders (e.g., epilepsy, schizophrenia, autism, anxiety and depression) (Altemus et al., 2014; Cozzoli et al., 2014; Hill, 2016; Kelley et al., 2011; Lovick, 2014; McHenry et al., 2014; Mendrek and Mancini-Marie, 2016; Reddy, 2014; Scharfman and MacLusky, 2014; van Luijtelaar et al., 2014), stress-related psychiatric disorders (Bangasser and Valentino, 2014), and addictive disorders (Fattore et al., 2014; Zhou et al., 2016). Neurodevelopmental disorders (Romano et al., 2016), autoimmune disease (Ngo et al., 2014), neurodegenerative disorders (e.g., Alzheimer's disease, Parkinson's disease, Huntington's disease, multiple sclerosis) (Gillies et al., 2014; Grimm et al., 2016; Kipp et al., 2016; Li and Singh, 2014; Litim et al., 2016; Mosera and Pike, 2016; Ramien et al., 2016), trauma (e.g., traumatic brain injury, spinal cord, stroke, post-traumatic stress disorder) (Gibson and Attwood, 2016; Inslicht et al., 2014), and diabetes (e.g., diabetic encephalopathy and peripheral neuropathy) (Lipscombe et al., 2014; Pesaresi et al., 2010; Zhao et al., 2014) also show sex differences in their incidence, symptoms and neurodegenerative outcome.

Interestingly, neuroactive steroid levels present in brain areas are also influenced by these pathologies in a sexually dimorphic way (Melcangi et al., 2016). Moreover, examples of sex specific effects of neuroactive steroids have been demonstrated, at least in experimental models (Mannix et al., 2014; Murray et al., 2003; Pesaresi et al., 2011a; Pesaresi et al., 2011b; Peterson et al., 2015; Zup et al., 2014). That is particularly interesting because may provide a possible background for a gender medicine based on these molecules to be applied in case of nervous pathologies (for a review see Porcu et al., 2016).

6. Conclusions.

Sex differences in the central and peripheral nervous system are widely diffused in vertebrates, in mammals and in humans. The mechanisms that cause the establishment of these dimorphisms may vary according to location and species, and involves several actors including: genes located in sexual chromosomes, epigenetic factors, gonadal hormones as well as neurosteroids. An increasing number of studies link human nervous pathologies with the

action of neuroactive steroids and with an effect of gender. Therefore, future research both in physiological and pathological conditions should consider more the interactions among genes, gonadal and brain-born hormones, epigenetic and environmental factors (as for example the exposure to endocrine disruptors, Frye et al., 2012), in order to have a more complex approach to the genesis of neural pathologies.

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