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

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ABSTRACT

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.

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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

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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 et al. (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 et al. 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, 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, 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