

Searching for Sex Differences in the Vomeronasal Pathway

Santiago Segovia and Antonio Guillamón

Departamento de Psicobiología, Universidad Nacional de Educación a Distancia, Ciudad Universitaria s/n, PO Box 60148 28040, Madrid, Spain

The sexual differentiation of brain and behavior is reviewed from the findings of sex differences in the vomeronasal pathway. A motivational approach to sex differences in reproductive behavior is stressed by taking into account that sex differences are present in neural networks: from the receptor organ (the vomeronasal organ) to effector nuclei. Sex differences in the brain appear in two morphological patterns. In one, the male presents greater morphological measurements than the female; in the other, the opposite occurs. These two morphological patterns are actively differentiated by gonadal steroids. The functional significance of these two morphological patterns is addressed. Moreover, since the GABA_A receptor is involved in the organization of sex differences in vomeronasal structures such as the accessory olfactory bulb and in maternal behavior, the role of membrane mechanisms, 5 α reduced hormones, and neurosteroids in the sexual differentiation process is discussed. © 1996

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Twenty-seven years ago, Phoenix, Goy, Gerall, and Young (1959) published a seminal paper entitled "Organizing Action of Prenatally Administered Testosterone Propionate on the Tissues Mediating Mating Behavior in the Female Guinea Pig." Let us quote three of the ideas expressed in this report: (1) "The embryonic and fetal periods are periods of organization or differentiation in the direction of masculinization or feminization. Adulthood, when gonadal hormones are being secreted, is a period of activation: neural tissues are the target organs and mating behavior is brought to expression" (p. 379). (2) "The suppression of the capacity for displaying the feminine components of the sexual behavior pattern which followed the administration of testosterone propionate prenatally appears to have been permanent" (p. 377). (3) "We are assuming that testosterone or some metabolite acts on those central nervous tissues in which patterns of sexual behavior

are organized. We are not prepared to suggest whether the site of action is general or localized" (p. 381).

The conceptual framework for posterior research on sexual differentiation in the nervous system and behavior is based on these three ideas. From these arose the concepts of "organization" and "activation." Only one question remained unanswered: whether the action of the gonadal steroids is general or localized on specific brain nuclei. The research done on sexual differentiation of the vomeronasal system (VNS) has supplied an answer to this question: the organizing action of the gonadal steroids affects complex neural networks involved in the control of reproductive behavior (Segovia and Guillamón, 1993).

REASONS FOR INVESTIGATING SEXUAL DIFFERENCES IN THE VOMERONASAL SYSTEM

At the end of the seventies, when we began to work on the sexual differentiation of the VNS, the research on sexual differences in the brain was focused on the preoptic area (POA) (Dörner and Staudt, 1968; Raisman and Field, 1971, 1973; Gorski, Shryne, Gordon, and Christensen, 1977; Christensen and Gorski, 1978; Gorski, Gordon, Shryne, and Southam, 1978) and the hypothalamus (see for review Dörner, 1976). Given that the POA and the hypothalamus control sexual behavior, it seemed natural that the search for anatomical differences between male and female brains begin in these regions.

Winans and Scalia (1970) and Scalia and Winans (1975) demonstrated the existence of a VNS or accessory olfactory system with a pattern of anatomical connections different from that found in the main olfactory system. Powers and Winans (1975), working with ham-

sters, found that the vomeronasal organ (VNO) is involved in the control of the male copulatory behavior. In later studies, it was proved that the attraction exerted by the female hamsters vaginal secretions on the male depended on the VNO input (Powers, Fields, and Wilians, 1979). Moreover, Johns, Feder, Komisaruk, and Mayer (1978) suggested that the VNO input could also play a part in the effect of pheromones on the regulation of the estrous cycle in the female rat. With regard to parental care, Fleming, Vaccarino, Tamboso, and Chee (1979) working with rats and Marques (1979) with hamsters demonstrated that the VNO also participates in the neural control of maternal behavior. These studies concerning the function of the VNO in reproductive behavior were a decisive factor in focusing our research on sex differences in the VNO.

At the beginning of the eighties we reported on the existence of sex differences in the rat's VNO and that this dimorphism depended on the organizational actions of the gonadal steroids during the immediate postnatal period (Segovia and Guillamón, 1982) and hypothesized that the VNS could be a sexually dimorphic neuronal network (Segovia and Guillamón, 1982, 1986). In later years we proved the existence of sex differences in the accessory olfactory bulb (AOB), the bed nucleus of the stria terminalis (BST), and the bed nucleus of the accessory olfactory tract (BAOT) (see, for review, Segovia and Guillamón, 1993; Guillamón and Segovia, 1993, 1996).

What have we learned in our search for sex differences in the VNS? (1) Mammals have sexually differentiated, complete, and complex neural networks. (2) There are two patterns of sex differences in mammalian brains: in one the male has greater morphological parameters in some nuclei than the female, and in the other pattern, the opposite occurs. (3) Changes in the permeability of neuronal membranes for ions could also be involved in the organization of the sex differences in the brain.

SEX DIFFERENCES APPEAR IN WHOLE NEURONAL NETWORKS IN MAMMALS

Our vision of the nervous system is rather Hebbian. Reproductive behaviors are so complex and their response patterns are so enchaind that it is impossible to understand their neurobiological control without basing them on neural networks.

Considering reproductive behavior as motivated behavior led us to try to explain sex differences in repro-

ductive behavior depending on sex differences in the VNS (Fig. 1). This implied taking into account the receptor organ and its anatomical projections. The results of the research carried out in our laboratory and those obtained by other authors (see, for review, Segovia and Guillamón, 1993; Guillamón and Segovia, 1996) support this approach to sexual differentiation of the nervous system and reproductive behavior.

THE TWO PATTERNS OF SEX DIFFERENCES IN THE MAMMALIAN BRAIN

While searching for sex differences in the BST we found that two subdivisions of the BST, the medial anterior (BSTMA) and the lateral anterior (BSTLA), did not have the typical pattern of sexual dimorphism, that is to say, a larger volume of the structure and a greater number of neurons in the male than in the female. In the BSTMA and the BSTLA, females show greater morphological measurements than males. These findings dissent from the known hormonal mechanisms for the organization of sex differences in the nervous system.

Pattern of Sexual Dimorphism in Which Males Show Greater Morphometric Measurements than Females

The male > female pattern depends on the known hormonal mechanism that is directly related to the organization concept proposed by Phoenix *et al.* (1959). According to this concept, testosterone induces the existence of more neurons and, as a consequence, a greater volume of the structures. This androgen-dependent hormonal mechanism involves the aromatization of testosterone to estradiol which is bound by intracellular receptors that regulate gene expression. Estrogens cause the sexual differentiation of the male and it was postulated that the estrogen-binding protein α -fetoprotein sequesters estradiol and protects the female brain from masculinization (Goy and McEwen, 1980).

Male orchidectomy or female androgenization during the critical period causes a decrease or an increase in the number of neurons and the volume of the structures, respectively. Moreover, the administration of estradiol to orchidectomized males during the period of maximum susceptibility counteracts the effects of castration. These facts have been proved in structures such as the VNO (Segovia and Guillamón, 1982), AOB (Segovia, Orensanz, Valencia and Guillamón 1984; Valencia,

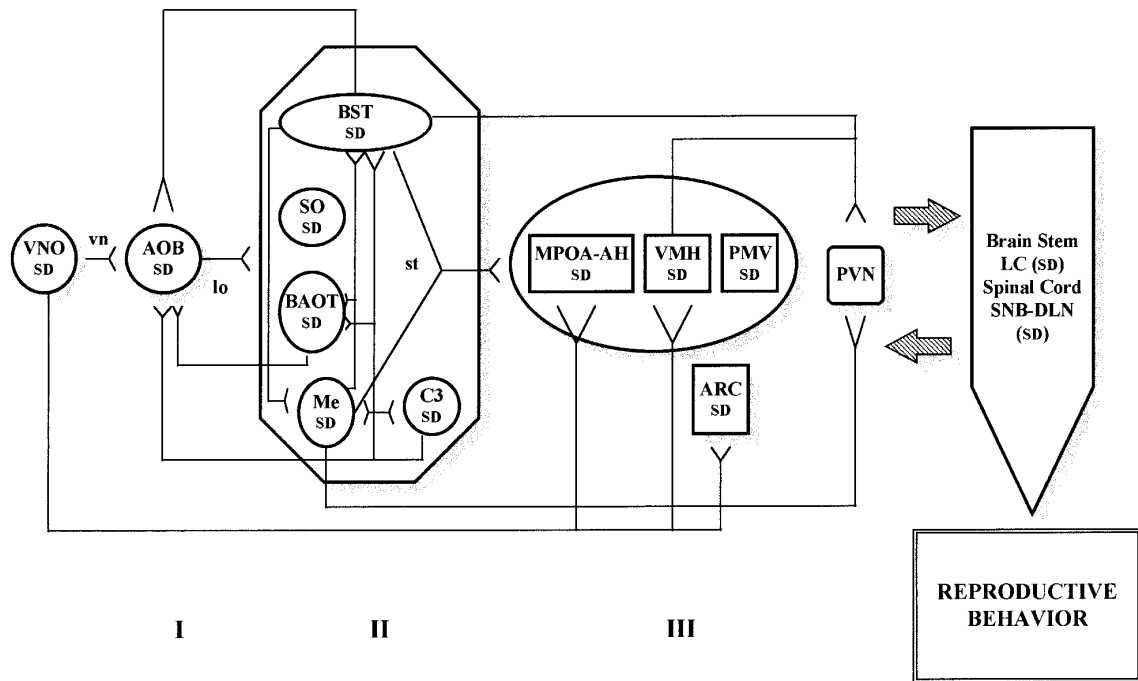


FIG. 1. An anatomical and functional (motivational) approach showing two sexually dimorphic networks (VNS-SNBS) that control sex differences in reproductive behaviors. I, II, and III, primary, secondary, and tertiary vomeronasal projections; SD, sex differences; AOB, accessory olfactory bulb; ARC, arcuate nucleus; BAOT, bed nucleus of the accessory olfactory tract; BST, bed nucleus of stria terminalis; C3, posteromedial cortical amygdaloid nucleus; LC, locus ceruleus; Me, medial amygdaloid nucleus; MPOA-AH, medial preoptic area-anterior hypothalamus; PMV, ventral premammillary nucleus of the hypothalamus; PVN, paraventricular nucleus of the hypothalamus; SNBS, spinal nucleus of bulbocavernosus system; SO, supraoptic nucleus; VMH, ventromedial hypothalamic nucleus; VNO, vomeronasal organ; lo, lateral olfactory tract; st, stria terminalis; vn, vomeronasal nerve (modified from Segovia and Guillamón, 1993). For vomeronasal connectivity, see De Olmos and Ingram (1972); Kevetter and Winans (1981); Larriva-Sahd, Rondán, Orozco-Estevéz, and Sánchez-Robles (1993); Simerly and Swanson (1986); Smithson, Weiss, and Hatton (1992); Winans and Scalia (1970); Scalia and Winans (1975). For connectivity between nuclei that receive vomeronasal input, PVN and SNBS, see Guillamón and Segovia (1996). Reprinted from *Brain Res. Rev.* **18**, Segovia and Guillamón, Sexual dimorphism in the vomeronasal pathway and sex differences in reproductive behaviors, 51-74 (1993), with kind permission from Elsevier Science-NL, Sara Burgerhartstraat 25, 1055 KV Amsterdam, The Netherlands.

Segovia, and Guillamón, 1986; Segovia, Valencia, Calés, and Guillamón, 1986; Pérez-Laso, Segovia, Collado, Rodríguez-Zafra, Del Abril, and Guillamón, 1996), the medial posterior subdivision of the BST (Del Abril, Segovia, and Guillamón, 1987a; Guillamón, Segovia, and Del Abril, 1988a), the BAOT (Collado, Guillamón, Valencia, and Segovia, 1990; Collado, Valencia, Del Abril, Rodríguez-Zafra, Pérez-Laso, Segovia, and Guillamón, 1993), the medial amygdala (Me) (Nishizuka and Arai, 1981a,b; Mizukami, Nishizuka, and Arai, 1983; Frankfurt, Siegel, Sim, and Wuttke, 1985; Micevych, Akesson, and Elde, 1988; Micevych, Matt, and Go, 1988; Malsbury and McKay, 1989), the sexually dimorphic nucleus of the preoptic area (SDN-POA) (Gorski *et al.*, 1977, 1978; Gorski, Harlan, Jacobson, Shryne, and Southam, 1980; Jacobson, Csernus, Shryne, and Gorski, 1981; Dölher, 1991), and the ven-

tromedial hypothalamus (VMH) (Matsumoto and Arai, 1983, 1986) (Table 1).

Pattern of Sexual Dimorphism in Which Females Show Greater Morphological Measurements than Males

This is a pattern of sex differences that has been known since early reports by Raisman and Field (1971, 1973). These authors described more nonamygdaloid synapses in the POA of the female rat as compared with the male. This sex difference was abolished by male castration at birth, which caused an increase in the number of these synapses, or the early androgenization of the female, which caused a decrease in the number of this kind of synapse. Matsumoto and Arai (1980) found the same pattern of sex differences with respect

TABLE 1

The Two Patterns of Sex Differences in the Mammalian Brain and Their Control by the Early Gonadal Environment^a

Structure	Pattern of sex differences	Neonatal male orchidectomy	Neonatal female androgenization	
VNO	Male > female	↓ ^b	↑	Segovia and Guillamón, 1982
AOB	Male > female	↓	↑	Segovia <i>et al.</i> , 1984, 1986; Valencia <i>et al.</i> , 1986; Pérez-Laso <i>et al.</i> , 1996; Collado <i>et al.</i> , 1990, 1993
BAOT	Male > female	↓	↑	Collado <i>et al.</i> , 1990, 1993.
BSTMP	Male > female	↓	↑	Del Abril <i>et al.</i> , 1987a; Guillamón <i>et al.</i> , 1988a
Me	Male > female	↓	↑	Nishizuka <i>et al.</i> , 1981a,b
NSD-POA	Male > female	↓	↑	Gorski <i>et al.</i> , 1977, 1978, 1980; Jacobson <i>et al.</i> , 1981; Dölher <i>et al.</i> , 1991
POA	Female > male	↑	↓	Raisman and Field, 1971, 1973
BSTMA	Female > male	↑	—	Del Abril <i>et al.</i> , 1987a,b
BSTLA	Female > male	↑	↓	Guillamón <i>et al.</i> , 1988a
PS	Female > male	↑	↓	Del Abril <i>et al.</i> , 1990
LC	Female > male	—	↓	Guillamón <i>et al.</i> , 1988b
ARC	Female > male	↑	↓	Matsumoto and Arai, 1980
AVPv	Female > male	—	↓	Arai <i>et al.</i> , 1993

^a Modified from Guillamón and Segovia, (1993, 1996).^b ↑, Morphometric increment; ↓, morphometric decrement; —, no effect.

to the number of spine synapses in the arcuate nucleus of the hypothalamus (ARC), and neonatal castration of male rats also caused an increase (“feminization”) in the number of spine synapses whereas neonatal androgenization of the females decreased the number of these synapses to the male level. Curiously, these findings did not attract a sufficient amount of attention for anyone to wonder about the hormonal mechanisms that control this pattern of differentiation.

As previously mentioned, we found a pattern of female > male in the volume and number of neurons of the BSTMA (Del Abril *et al.*, 1987a; Del Abril, Guillamón, and Segovia, 1987b) and the BSTLA (Guillamón *et al.*, 1988a). Neonatal orchidectomy of males increased the volume and number of neurons of the BSTMA (Del Abril *et al.*, 1987a,b). Androgenization of the female on the day of birth reduced the number of neurons in the BSTLA, whereas orchidectomy on the day of birth increased the number in male rats (Guillamón *et al.*, 1988a). An analogous pattern was found in the parastrial nucleus (PS) (Del Abril, Segovia, and Guillamón, 1990) and in the locus ceruleus (LC) (Guillamón, De Blas, and Segovia, 1988b) which projects to VNS structures (Swanson and Hartman, 1975; Saper, Swanson, and Cowan, 1976). In the PS and LC, the females had a greater volume and/or number of neurons than the males. Male orchidectomy on the day of birth caused an increase in the PS volume, whereas the androgenization of the female at birth induced a decrease (Del Abril

et al., 1990). Male castration did not increase the volume or number of neurons in the LC, whereas androgenization of the female caused a decrease in these measurements (Guillamón *et al.*, 1988b). Arai, Nishizuka, Murakami, Miyakawa, Machida, Takeuchi, and Sumida (1993) reviewed the development of sex differences in the anteroventral periventricular nucleus of the POA (AVPv). The AVPv shows a female > male morphological pattern and testosterone or estrogen neonatal treatments caused a significant decrease in AVPv volume in the female rat (Table 1).

The results of these investigations led us to suggest that when the female > male pattern appears, the differentiation of the female depends on estrogens, and testicular androgens, far from having a trophic activity, have an “inhibitory” influence on the male (see, for review, Segovia and Guillamón, 1993; Guillamón and Segovia, 1993, 1996). The existence of two morphological patterns in both male and female brains indicate that male and female are two different neurobehavioral dimensions.

FUNCTIONAL SIGNIFICANCE OF THE QUANTITATIVE SEX DIFFERENCES IN THE VOMERONASAL PATHWAY

Another question that arises concerns what the functional significance of these two patterns of sex

differences might be. To approach this problem we used two simple propositions (Segovia and Guillamón, 1993). First, when one sex has more neurons in a nucleus than the other, these are considered "extra" or supernumerary neurons that contribute to behavioral sex differences possibly involving that specific nucleus. Second, most of the experiments performed to determine the functional role of a VNS structure use lesion, resection, or stimulation techniques. Using these techniques we can determine if a particular structure has a facilitatory or inhibitory role on reproductive physiology and behavior. Since these techniques suppress or activate neurons, the results from these studies could be considered analogous to those produced when there is a change in the number of neurons of a sexually dimorphic nucleus.

Working with these two propositions we hypothesized (Segovia and Guillamón, 1993) that in the male > female pattern the supernumerary neurons in the male might be related to the facilitation of masculine sexual behavior and, what might be most important, to the tonic inhibition of the display of lordosis and maternal behavior. This has been especially proven in the BSTMP after electrolytic lesions that impair male sexual behavior (Claro, Segovia, Guillamón, and Del Abril, 1995). Regarding maternal behavior, electrolytic lesions of the BAOT facilitate maternal behavior in virgin female rats as well as males (Del Cerro, Izquierdo, Collado, Segovia, and Guillamón, 1991; Izquierdo, Collado, Segovia, Guillamón, and Del Cerro, 1992). In addition, neonatal administration of the GABA_A agonist diazepam (DZ) to males was able to induce a female-like pattern in the number of AOB mitral cells and maternal behavior, whereas the antagonist picrotoxin (PTX) neonatally administered to females caused a male-like pattern in the number of AOB mitral cells and disrupted maternal behavior. Interestingly, neither DZ nor PTX caused any alterations in the plasma levels of the gonadal hormones (Segovia, Del Cerro, Ortega, Pérez-Laso, Rodríguez-Zafra, Izquierdo, and Guillamón, 1996).

On the other hand, the female > male pattern would be related to the nonreceptive phase of the estrous cycle in the female (Segovia and Guillamón, 1993). Electrochemical stimulation of the lateral division of the BST on the day of proestrus prevents the preovulatory discharge of luteinizing hormone in the female rat (Beltramino and Taleisnik, 1980). It should be remembered that the BSTLA has a pattern of sex differences in which the female shows greater volume and number of neurons than the male (Guillamón *et al.*, 1988a).

ARE CHANGES IN THE PERMEABILITY OF NEURONAL MEMBRANES TO IONS INVOLVED IN ORGANIZING BRAIN SEX DIFFERENCES?

The concept of organization establishes that the gonadal hormones differentiate the neural tissue and reproductive behavior during an early period of the development, whereas these hormones have an activating function on those neural tissues during adulthood (Phoenix *et al.*, 1959). The genomic action of the steroid hormones was coherent with the organizational function that takes place during perinatal development given that the steroid action at this time is permanent and irreversible. The so-called nongenomic or membrane actions of the steroids were associated with the activation function, because they are rapid and reversible and have been studied during adulthood. The association of these ideas (organization–genomic actions vs activation–membrane actions) has undergone changes and this dichotomy has been seriously questioned (Arnold and Breedlove, 1985). It is well known that genomic actions also take place in the adult period and that they participate together with the nongenomic actions in controlling reproductive physiology and behavior (Arnold and Breedlove, 1985; McEwen, 1991a,b; García-Segura, Chowen, Párducz, and Naftolin, 1994; McCarthy, 1995). However, the question now is whether the changes in the permeability of the neuronal membrane to the ions are involved, together with the genomic actions, in the organization of brain sex differences.

Role of the GABA_A Receptor in the Organization of Brain and Behavioral Sex Differences

The "cascade" hypothesis (Toran-Allerand, 1984) indicates that testosterone and estradiol cause a series of enchain events whose consequence is the sexual dimorphism that is observed in the nervous system regarding the number of synapses and neurons, the volume of structures, and neurite size. In this hypothesis the synapses play a major role in explaining sexual dimorphism in the number of neurons and, consequently, in the volume of the structures. The neurotransmitters are relevant to the cytoarchitecture of the brain (Mattson, 1988; Lipton and Kater, 1989), and for this reason, investigations on studied the influence of hormones and neurotransmitters in sexual differentiation of the mammalian hypothalamus were carried out (Dölher, 1991).

We have studied the involvement of the main inhibitory neurotransmitter in the brain, GABA, in the development of sex differences. In 1991 we reported evidence that the GABA_A agonist DZ, administered from the day of birth (P0) to P16, decreased the AOB volume and the number of AOB mitral cells inducing a female-like neuromorphological pattern in this VNS structure of the male, whereas this treatment did not significantly affect the same structure in female rats (Segovia, Pérez-Laso, Rodríguez-Zafra, Cales, Del April, De Blas, Collado, Valencia, and Guillamón, 1991). Moreover, perinatal DZ treatment also caused female-like organization in the AOB of males without affecting females (Pérez-Laso, Valencia, Rodríguez-Zafra, Cales, Guillamón, and Segovia, 1994). Similar results were reported in the SDN-POA of the male rat using the agonist muscimol (Bach, Flugge, and Wuttke, 1992).

Recently, we investigated (Segovia *et al.*, 1996) the role of the GABA_A receptor in the development of sex differences in the AOB and in parental behavior. The agonist DZ and the antagonist PTX were administered from P0 to P16 to male and female rats, respectively. We found that DZ was able to induce a female-like number of AOB mitral cells and maternal behavior when they were adults, whereas PTX masculinized the AOB in females and disrupted their maternal behavior. Interestingly, measures of the levels of estradiol, testosterone, and progesterone in plasma performed immediately after the drug treatments and after the maternal behavior test remained unaltered in each sex. Brain sex and behavioral sex were changed by the administration of DZ and PTX, but hormonal sex was not. This suggests that the alterations observed in sex differences at the behavioral level in the adult animals may be due to early postnatal changes in the permeability of neuronal membranes to the Cl⁻ ion. These changes in Cl⁻ permeability altered the number of mitral cells in the AOB and could have caused an "imprinting" in the physiology of the GABA_A receptor.

Studies performed in cortical cultures have shown that GABA accelerates excitotoxic cell death induced by excitatory amino acids, whereas blockers of the GABA-gated Cl⁻ ion channel protect against excitotoxicity (Erdö, Michler, and Wolff, 1991). An analogous process might be associated with the reversal of sex differences in AOB mitral cells that we observed after the DZ and PTX treatments. In addition, it has been reported that a single administration of DZ causes a significant increase in the 5 α -reductase activity in the diencephalon of the rat without altering plasma testosterone levels (Kaneyuky, Kohsaka, and Shohmori, 1979). We suggest that possible participation of 5 α -reduced nongonadal

origin steroids should be taken into account in explaining the results we obtained concerning the organization of sex differences in the AOB and maternal behavior (Segovia *et al.*, 1996), and that perhaps this mechanism could also be involved in the sexual differentiation of the brain and behavior.

The fact that the early postnatal administration of an agonist and an antagonist of the GABA_A receptor, known to alter Cl⁻ flux in this receptor, changes the neural sex in the AOB and the behavioral sex in maternal behavior, without causing significant changes in the plasma levels of gonadal hormones, suggests that mechanisms directly related to changes in the permeability of neuronal membranes might participate, together with the genomic mechanisms, in the organizational process of sex differences. Moreover, this constitutes a new animal model that might contribute to an understanding of the early postnatal differentiation of the brain when genetic sex and hormonal sex do not match brain sex and behavioral orientation in adulthood.

Here it is also important to mention that in a structure like the LC in which the sex difference pattern is female > male, the neonatal administration of DZ inverted this morphological pattern, causing a decrease in the volume and number of neurons in the female without affecting the male (Segovia *et al.*, 1991).

Are Neuroactive Steroids that Modulate the GABA_A Receptor Involved in Early Organization of Sex Differences?

The GABA_A receptor is modulated by the steroid hormones, particularly by the 5 α reduced metabolites of progesterone, corticosterone, and deoxycorticosterone. The report by Majewska, Harrison, Schwartz, Barker, and Paul (1986) is key to understanding how the GABA_A receptor is modulated by these steroids. The neuroactive steroids, including the neurosteroids, are capable of regulating the channel function of several ions, such as Cl⁻, K⁺, and Ca²⁺, and are implicated in the control of adult reproductive behavior (Beyer and González-Mariscal, 1991; Majewska, 1992; Paul and Purdy, 1992; Lambert, Belelli, Hill-Venning, and Peters, 1995; McCarthy, 1995; Karst and Joéls, 1996). The GABA_A receptor is a target of the actions of the neurosteroids (Baulieu and Robel, 1990; Majewska, 1992; Lambert *et al.*, 1995), which are synthesized in the brain (Corpéchet, Robel, Axelson, Sjövall, and Baulieu, 1981; Hu, Bourreau, Jung-Testas, Robel, and Baulieu, 1987).

Two genes encode two 5 α -reductase isozymes, type I and type II, that differ in amino acid sequence (60% of

identity), optimum pH, and sensitivity to the inhibitor finasteride (type I is inhibited weakly by finasteride, whereas type II is sensitive to this potent inhibitor) (see, for review, Russell and Wilson, 1994). The 5α -reductase system is widely distributed throughout the CNS and highly concentrated in the white matter (Celotti, Melcangi, and Martini, 1992). The mRNA type I 5α -reductase is detected in the brain as early as Prenatal Day 14 (E14) and is constantly expressed during development and adult life (Poletti, Negri-Cesi, Rabuffetti, Celotti, and Martini, 1996). However, in the brain of the rat the type II 5α -reductase isozyme is undetectable from E14 to E16, and progressively increases after E18, showing a peak of maximal expression on P2 and decreasing until becoming undetectable on P28 (Poletti *et al.*, 1996).

The participation of the 5α -reduced metabolites of progesterone (Beyer and González-Mariscal, 1991) and the 5α -reductase type II isozyme (Poletti *et al.*, 1996) has been postulated in the sex differences in the brain. In addition, since neonatal modulation of the GABA_A receptor causes a reversion in the sex differences at the neural and behavioral levels without affecting gonadal function (Segovia *et al.*, 1996) it has also been suggested that the neurosteroids and the 5α -reduced metabolites are involved in the organization of sex differences in brain and behavior and that the GABA_A receptor could act in this process as a common final pathway (Segovia *et al.*, 1996).

All of this gives rise to four new questions concerning sex differences in the brain and behavior: (1) Aside from the known effects of activation in adults, do the 5α -reduced steroids participate in the organization of sex differences? (2) If the answer to (1) is yes, what is the function of the two 5α -reductase isozymes? (3) Keeping in mind that the activity of the acyl-transferase enzyme is particularly high during the first 21 postnatal days (Baulieu and Robel, 1990) do the neurosteroids play a significant role in the organization of sex differences? (4) With what do the two 5α -reductase isozymes interact and what is their interaction with the two separate aromatase-immunoreactive systems found in the rat brain (Jakab, Horvath, Leranath, Harada, and Naftolin, 1993; Naftolin, 1994) when it comes to creating an adequate steroid environment in the brain that results in sex differences? In the coming years the answers to these questions could provide a clearer picture of the extremely complex process of brain and behavioral sexual differentiation.

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