

REVIEW ARTICLE

# Structural sex differences in the brain: Influence of gonadal steroids and behavioral correlates<sup>1</sup>

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## INTRODUCTION\*

Considerable debate exists in the contemporary scientific literature regarding the origin and existence of sex differences in the behavior of women and men (1). Recently, evidence was found for the existence of DNA loci that may relate to sexual orientation in man (2,3). A number of other studies [for reviews see (4-6)] have attempted to establish a biological basis for gender differences in humans. However these studies are often inconclusive, due in part to the tremendous variability and role of learning.

Sexually dimorphic behaviors, particularly those important in reproduction are very evident when considering animal models. Historically, there was debate concerning the possibility that both sexes were capable of showing male- and female-typical behavior, with testicular steroids eliciting male-typical behavior and ovarian steroids stimulating female-typical behavior. Further, gonadectomy during the prepubertal period resulted in a loss of mating behavior, which was restored by replacing the appropriate hormone or some of its relevant metabolites (7,8). More recent investigations have demonstrated that sex differences in behavior rely on neonatal exposure to gonadal steroids and the later presence of appropriate gonadal steroids in the adult for full expression of sexual behavior (9-14). Conversely, alterations that occur in neuroendocrine systems during aging, particularly relative to the effects of loss of estrogen on the function of the female hypothalamic neuroendocrine systems also provide new insights into how steroid modulate neuronal circuitries. However, it has to be pointed out that a

coverage of the wide literature on aging of neuronal systems involved in the control of reproduction is beyond the scope of the present paper, and the reader is addressed to some other recent reviews (5,15-17) for additional comments. In addition, we will give a few example of effects of aging on some well defined sexually dimorphic structures.

## ACTION OF STEROID HORMONES ON THE VERTEBRATE BRAIN

The presence of gonadal steroid receptors within the central nervous system (CNS) of vertebrates has led to hypotheses of specific actions of these steroids in differentiation and the postnatal development of target cerebral circuits, as well as in the maintenance of their functions in the adult brain (18,19). As a result, a great deal of research was conducted during the 1970s and the 1980s in which structural sexual dimorphisms were characterized in the CNS. In a strict sense the term dimorphic should be used only in presence of large differences in the shape of considered structures, however, in the following text we will use the terms sexual difference and sexual dimorphism as interchangeable because in some instances they will be referred for quantitative differences that are rather small and other times for qualitative differences that are substantial. Frequently, the dimorphic structures and the receptors for gonadal steroids were found in the same or in closely related regions, providing indirect evidence that the origin and the presence of these neuroanatomical dimorphisms might be determined by gonadal steroids. A more convincing demonstration of this putative role of gonadal hormones has been recently reached by the administration of antisense oligoprobes to estrogen receptor (a technique that blocks the target protein synthesis). When administered during a sensitive developmental period they prevent the establishment of the sexual dimorphism in the rat preoptic region (20). Some mechanisms of steroid hormone action initially

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<sup>1</sup>Invited lecture at the XV Congress of the Spanish Anatomical Society, Puerto De La Cruz, Tenerife, December 1993.

<sup>2</sup>Scientific Article n.6579, Contribution n.8791 of the Maryland Agriculture Experiment Station (Department of Poultry Science).

*Key words:* Sexual dimorphism, sexual behavior, androgens, estrogens, estrogen receptor, hypothalamus, preoptic area, song control nuclei, central nervous system, vertebrates.

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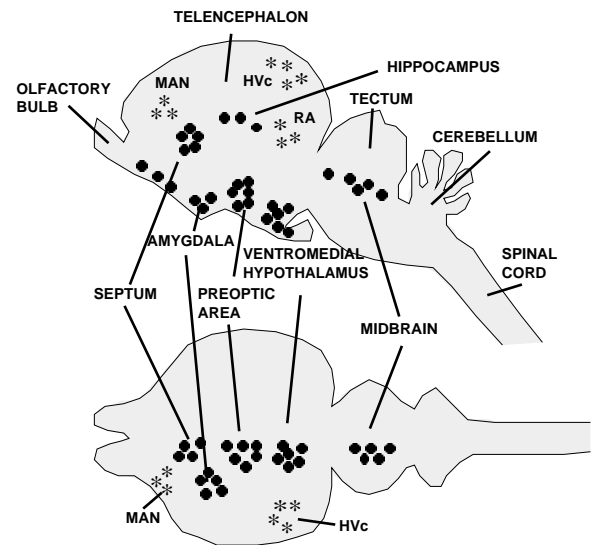
investigated non-neural target organs and only subsequently adapted to the brain. Some of the components established as part of steroid hormone action included binding of the activated hormone-receptor complex to intranuclear acceptor sites, subsequent production of messenger RNA followed by protein synthesis (21). Such studies on non-neuronal tissues also led to the identification of cell-specific proteins that have been induced by the action of steroids (22).

Unfortunately, the anatomic and functional heterogeneity of the nervous tissue has complicated the possibility of isolating unique products (23,24). However, both anatomical and biochemical approaches have been utilized in the determination of steroid hormone sensitive regions of the brain. Mapping the location and distribution of steroid-concentrating neurons has been accomplished in many vertebrate species through the use of receptor autoradiography [for review, see Warembourg (25)]. These studies have established that receptor sites for estrogens, androgens, progestins, glucocorticoids, and mineralcorticoids exist in a phylogenetically stable manner in various regions of the brain [for a review, see McEwen et al. (18)]. The most numerous targets for steroid hormones appear to be neurons within the hypothalamus and the limbic region (Fig.1). The recent development of immunocytochemical reagents to detect estrogen, and androgen receptors (26-30), as well as other molecular techniques, such as *in situ* hybridization for the detection of the corresponding messenger RNA (31), have provided confirmation of the steroid receptor distribution described in early autoradiographical studies. Moreover these recent studies indicate also that species differences in sexual steroid sensitive behavior could be encoded in the neural gonadal steroids receptor distribution (30). This possibility is obvious for the songbird forebrain and the singing of songbirds (Figs 1, 4).

Steroids exert their physiological effects on the brain in different ways. These effects may be broadly classified in long-term or short-term effects on the basis of their latency and duration [for a review see Fink et al. (32)]. Long-term effects last for many days, weeks, or even lifetime; hence they can be further subdivided in reversible or irreversible. The short-term effects take either only minutes (or seconds) to develop (rapid effects), or much longer time (intermediate effects) and may last for several hours. There are many examples of long-term reversible effects of steroids on the vertebrate central nervous system. Further, the nature of the effects of steroids on specific brain nuclei may occur in terms of volume, shape, cytoarchitecture, or innervation and depend on the availability of gonadal hormones (33-36). Often these types of effects have been broadly described as *activational* effects. This refers to the observation that neural pathways, regardless of sexual differentiation, can be either stimulated or depressed by the presence of the appropriate steroid hormone.

Long term irreversible (currently called *organizational*) effects of steroids are those instrumental in the determination of the constitution of dimorphic

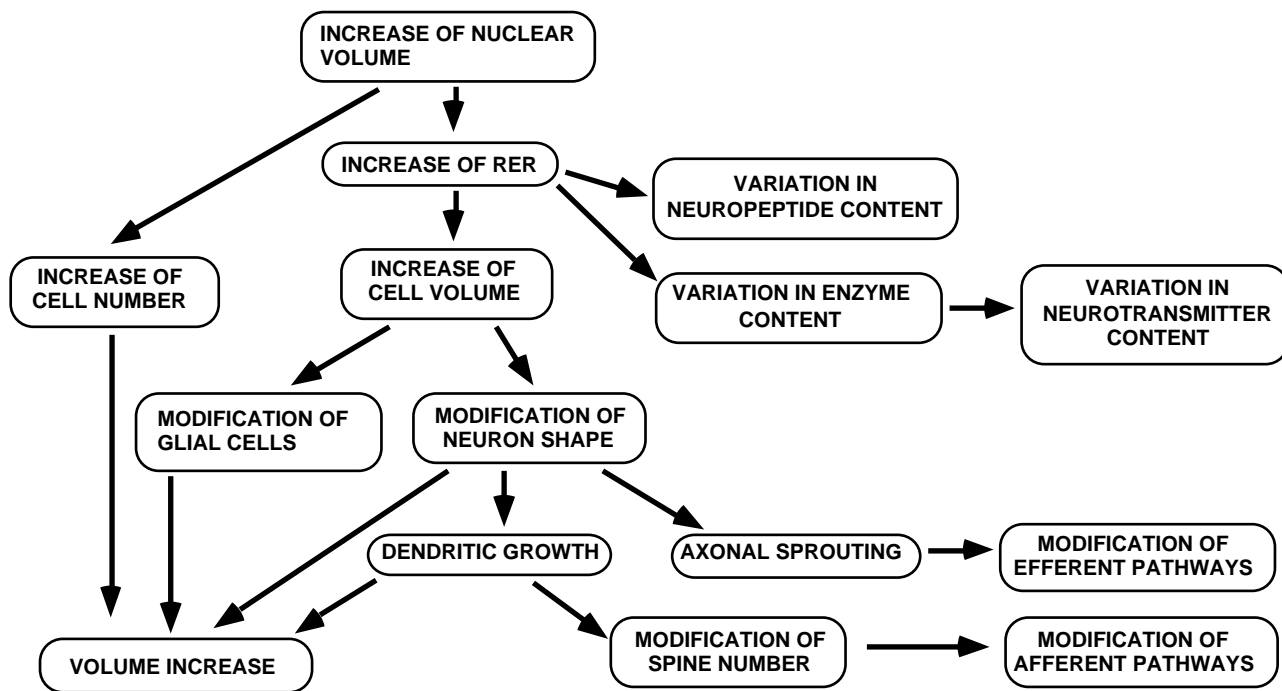
pathways. An obvious example is the action of testosterone (T) in masculinizing the brain; this process will be further discussed in subsequent sections. For further discussions concerning the classification of different effects of steroids see also the review of Arnold and Breedlove (37).



**Fig. 1.** Distribution of estradiol receptors in the vertebrate brain. Large black dots represent the the main centers which are commonly present in all vertebrates. Stars approximately indicate the presence of estradiol receptors in avian oscine (singing) species. MAN: magnocellular nucleus of the anterior neostriatum; Hvc: caudal nucleus of the hyperstriatum ventrale; RA: robust nucleus of the archistriatum.

The mechanism of gonadal hormone action within nerve cells includes the migration of the complex hormone-cytoplasmic receptor to the nuclear compartment, the binding of the activated hormone-receptor complex to genomic acceptor sites, and the subsequent activation of a sequence cascade [Toran-Allerand (38)]. Following activation via this sequence cascade, there are numerous morphological effects that may ensue. Some of these responses are listed below and reported in Fig. 2:

- a) modification of nuclear size,
- b) rearrangement of the ultrastructure of cellular compartments related to protein synthesis,
- c) changes in neuronal enzyme levels,
- d) modification of cell body size or shape,
- e) variations in the rate of synthesis of neurotransmitters or neuropeptides,
- f) alteration of the efferent circuits,
- g) sprouting of axonal projections,
- h) growth of dendritic arborization with increased number of spines,
- i) modification of afferent circuits,
- l) altered glial elements,
- m) increase in cell number
- n) volumetric changes of neuronal aggregations (neuronal nuclei).



**Fig. 2.** Sequence of morphological phenomena (cascade) following the activation of a steroid sensitive neuronal system.

Of the effects of steroid hormones mentioned above, the regulation of the number of cells in a selected region is one of the most significant to determine volumetric changes in the brain. Three general mechanisms could account for this effect (39,40): (1) the steroid hormone could stimulate neurogenesis in one sex, or (2) could prevent neuronal death, or (3) could regulate processes influencing the differentiation of neurons. In a review concerning the avian song control nuclei Arnold and coworkers (39) reported data suggesting that the mechanism of regulation of neuron number by estradiol is not univocal and may differ according to the various telencephalic nuclei.

The aforementioned sequence of events does not require hormone involvement at every point. For example, the formation of synaptic contacts on the dendritic tree (whose growth was stimulated by the gonadal hormone) might be influenced by some other environmental factors. Moreover, the hormone might have a trophic effect on a group of neurons that could cause enhanced innervation of its target(s). This in turn would have anterograde effects on neurons in those regions, in a kind of domino theory (41). Therefore, the morphology and connections of a specific brain region is the result of an interaction of hormone-dependent and hormone-independent entities [Tobet and Fox (42)]. It is also important to mention that, in recent years, several experimental papers have suggested the possibility that the rapid effects of steroids depend on a direct membrane effect (43,44). For example a considerable body of evidence has accumulated for action of certain

steroids on the chloride channel of the GABA<sub>A</sub> receptor (45) or of the progesterone on the  $\alpha_1$ -adrenergic-dependent cyclic AMP formation (46). These rapid effects may also involve structural reorganization of the postsynaptic membrane: for example the increase in the number of pits in the post-synaptic membrane of *in vitro* incubated arcuate nucleus slices reached its plateau at 1 minute following addition of estradiol (47). A recent report suggests that a corticosterone receptor localized to synaptic membranes may exert short latency inhibitory effects on male sexual behavior in amphibians (48). It has been recently demonstrated that estrogen may facilitate in short term (2 hours, i.e. not long enough for genomic effects) basal and noradrenaline-stimulated release of LHRH from quail hypothalamic slices (49). It is dubious whether this responsiveness is due to a direct action of estradiol on the peptidergic neurons or whether the steroid acts on neighbouring regulatory systems. In conclusion, non-genomic effects of steroids should be considered as an important additional mechanism modulating the actions of slower and more permanent genomic effects.

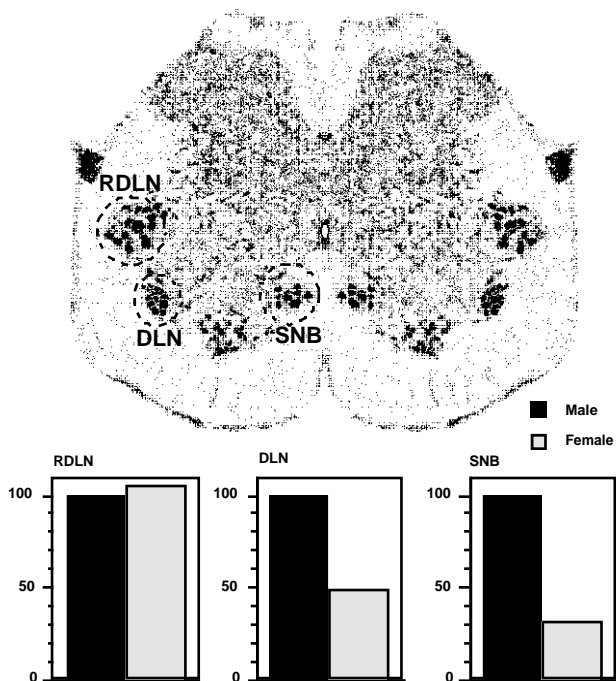
### SEX DIFFERENCES IN THE CENTRAL NERVOUS SYSTEM

Sexually dimorphic nuclei or regions have been described in several species of tetrapods and have been previously reviewed [for a more extensive recent list of references, see Tobet and Fox (42), Breedlove (50)]. These nuclei have been observed in several contexts including: 1) neuronal groups directly related to peripheral

dimorphic organs [i.e. motoneurons of the spinal cord controlling the penis muscles (51,52) or motoneurons controlling the avian syringeal musculature (53,54)]; 2) cerebral nuclei without any evident connection to sexually dimorphic structures or organs [i.e. the dimorphic nuclei of the preoptic region in many vertebrate species (36,55-62) including man (63,64), or the accessory olfactory pathway in the rat (65)]. Some of these examples will be discussed further below.

*Spinal cord motor neurons*

Certain groups of mammalian spinal cord motor neurons exhibit a simple sexual dimorphism. In the fifth and sixth lumbar segments of the male rat spinal cord are two motoneurons cell groups that innervate the muscles of the penis and the perineal region (Fig. 3).



**Fig. 3.** Sexual dimorphism in the spinal cord motor neurons. The drawing represent a cross section of the lumbar spinal cord of an adult male rat [according to Jordan et al. (66)]. Circles identify three motoneuronal groups: RDLN (retrodorsolateral nucleus), DLN (dorsolateral nucleus), and SNB (spinal nucleus of the bulbocavernosus). The histograms at the bottom report sexual differences in the number of motoneurons within these nuclei. The number in male was conventionally put to 100. Only SNB and DLN show sex dimorphism [data according to Breedlove and Arnold (51,52), Jordan et al. (66)].

The first group includes the neurons innervating the bulbocavernosus and levator ani muscles (spinal nucleus of the bulbocavernosus, SNB). The second group includes the neurons innervating the ischiocavernosus muscle (dorsolateral motor nucleus, DLN). Normally these muscles and the SNB are absent in the female, whereas a DLN only is present in females, where it contains fewer and smaller neurons than in males (66). Several experimental studies have established that perinatal treatment of females with

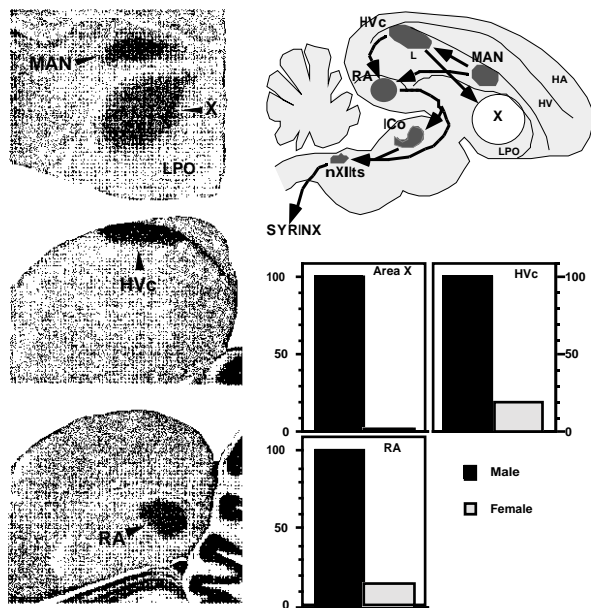
androgen will exert permanent effects on the survival of both spinal neurons and of their muscular target (67,68). It appears that androgen acts directly on the muscles, preventing their degeneration. The survival of DLN and SNB neurons is a secondary response to the survival of their muscles [Forger et al. (69) for a review]. Size and ultrastructural organization of both SNB and DLN neurons are affected in the adult male by the deprivation of T (51,70-72). Homologous sex-dependent spinal motor nuclei also are found in dogs and humans (73).

The model of the spinal neurons controlling the penis musculature has also been useful in our understanding the relationship of a nervous sexually dimorphic structure and its target, as well as the developmental effects of gonadal hormones. In this case the structural dimorphism (number and size of neurons) of neuronal centers might be considered as a consequence of the dimorphism of its target tissue. This is probably a relatively simple model for the elucidation of more elaborate circuits and behaviors. In contrast, sexual dimorphisms in telencephalic or diencephalic regions may represent a morphological substrate which facilitates complex behavioral differences in the two sexes.

*Nuclei controlling avian vocal behavior*

A typical example of a complex sexually differentiated behavior is the avian singing behavior. In songbirds (oscine: passeriformes) male song is often more complex than female song. Females show the range of song from a total absence (zebra finch) to infrequent and less complex song (canary, white-crowned sparrow). Songs in these species represent a powerful sexual display and play a critical role in courtship (74). Nottebohm and cowoekers (75,76) demonstrated a neuronal pathway starting from the telencephalon, passing through mesencephalic stations and ending at the XII nerve nucleus (controlling the musculature annexed to the syringe, and hence the structure of the call). This pathway exhibits both gonadal steroid receptor-bearing neurons (77) and a volumetric sex difference in many of the telencephalic nuclei, with the area generally larger in males than in females (75,78) (Fig. 4). Moreover, some of these nuclei are not only sexually dimorphic, but they show seasonal changes directly related to variations in the singing behavior (79). Changes in the adult song system of oscines are species specific, in fact canaries show marked seasonal changes in the adulthood (36,79) but these changes do not occur in zebra finch brain (80).

The vocal control system of birds has proven to be a useful model for correlating structure (nuclear volume, neuron morphology) and function (ability to sing), including correlations of species-specific differences in song production and perception with variations in nuclear volume (39,81).



**Fig. 4.** Song controlling system in songbirds. Drawings on the left show the location and extension of telencephalic sexually dimorphic steroid-accumulating nuclei in male zebra finch. MAN: magnocellular nucleus of the anterior neostriatum; X: area X; HVC: caudal nucleus of the hyperstriatum ventrale; RA: robust nucleus of the archistriatum; LPO: paraolfactory lobe [drawings are from sections reported by Arnold (78)]. Top right: schematic diagram of brain regions involved in song control in passerine birds [according to the data of Nottebohm et al. (76)]. Projections are indicated by arrows. ICo: intercollicular nucleus; nXIIIts: tracheosyringeal portion of the hypoglossal motor nucleus; L: field L; HA: hyperstriatum accessorium; HV: hyperstriatum ventrale. Steroid-receptor containing nuclei are marked by dots. The histograms show the sexual dimorphism of volume of some telencephalic nuclei of the system (Area X, HVC, RA) in Zebra finch. The volume of male nuclei were conventionally put equal to 100. Data are from Arnold (78).

Recent studies have also pointed to the important relation of auditory pathways and the song control system. Further, there are remarkable differences found in various avian species (e.g. the canary, the zebra finch, and the budgerigar) both in the arrangement of telencephalic nuclei, and in their connectivity (82-85).

In galliform species (i.e. in non-singing species), attention has recently focused on the mesencephalic nucleus [nucleus intercollicularis, ICo (86)] involved in the control of male (and/or female) vocalizations. Both estrogen and androgen receptors have been localized in the quail ICo (30,87,88) particularly in the dorsomedial part of this complex nucleus. Neurons in this region are relatively small in pre-puberally gonadectomized quail and enlarge in response to T treatment only in male (89). In contrast, these cells are responsive to T administration in both sexes in the gray partridge (90). It is important to emphasize that in non-oscine birds no specialized telencephalic

structure comparable to the oscine nuclei has been so far described.

In summary, results from these studies on avian telencephalic song nuclei support a dual role for T. During the critical period of sexual differentiation in the zebra finch song system, T may act as a prohormone, serving as a precursor for estradiol and dihydrotestosterone formation (91-93). In the adult, T is likely to play a critical role in the activation of reproductive responses. In canaries, there appears to be a direct action of T through androgen receptors as well as through its metabolites (94).

#### The vertebrate preoptic region

The preoptic area (POA) and the hypothalamus regulate both endocrine and behavioral components of reproduction [i.e. the hypophyseal secretion of gonadotrophins, the mount and lordosis behavior (95)]. Moreover, steroid receptors are found in the anterior hypothalamus (including the preoptic region) and in tuberal region of virtually all vertebrates (18). Physiological, biochemical and immunocytochemical studies have demonstrated that many aspects of the POA function are sexually differentiated. Steroid action in the POA is sexually dimorphic as revealed in mammals by differential concentrations (96,97) or differential inducibility of receptors in males versus females (98,99). In birds, there are sex differences in the metabolism of T (100-103). During the last decade, many studies in mammals and other vertebrates have focused on the problem of sexual dimorphism in brain morphology. Within the POA, sex differences have been identified in neuronal connectivity, cell nucleus size and in the volume of groups of cells. These differences fall into 5 main categories:

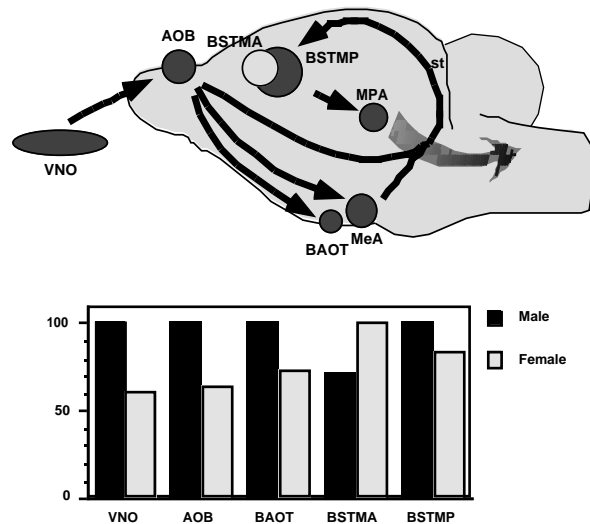
- 1) Differences in the gross volume of defined cell groups of the septo-preoptic region as shown in several mammals [rat (55), guinea pig (56), ferret (33), gerbil (57,104), and also man (63,64)], in birds [Japanese quail (58)], in lizard (61), and in the toad [*Bufo japonicus*, (60)],
- 2) differences in the shape of hypothalamic nuclei, as shown in the rat supra-chiasmatic nucleus (105),
- 3) differences in the dendritic organization of the preoptic region in the monkey (106),
- 4) ultrastructural differences in synaptic organization as demonstrated in the preoptic region of the rat (107), and
- 5) differences in the distribution of various peptidergic or neurotransmitter systems [vasopressin (108), cholecystokinin (109), substance P (110,111), serotonin (109), for review, see De Vries (112)].

Generally these structural differences are steroid dependent for their development and maintenance. The pioneer experiments of Raisman and Field (107) demonstrated that some pathways within the POA of the rat are steroid sensitive. These experiments showed that

perinatal steroid treatment of rats resulted in a modification in the number and types of axo-dendritic synapses in a specific part of the POA as well as permanent and irreversible changes in the sexual behavior of the rat. Moreover, these data demonstrated a critical perinatal period in which irreversible determination of sex-related behavioral responses and structural dimorphic characteristics occur. However, due to the long work involved, these results generated a great interest but only a limited number of researches. The studies of Gorski et al. (55,113) showing that a sexually dimorphic structure can be detected in the mammalian brain at the light microscopic level was not only an important contribution in our understanding of the action of steroid hormones at the hypothalamic level, but also, together with the pioneering work of Nottebohm and Arnold (75) on avian song control nuclei, a more important conceptual approach to the study of neural sexual dimorphism. In that study, the authors demonstrated a sexually dimorphic part of the medial preoptic nucleus, which is larger in male than in female rats. This cell group was initially termed the sexually dimorphic nucleus of the POA (SDN-POA). The volume of this structure proved to be sensitive to perinatal T treatment in the female. In fact, volume of this area was found to increase to a size similar to that of the male when the female was implanted with T propionate during the first week of life. These data provided evidence for the irreversible nature of male-typical behavior in treated females. Sexually dimorphic structures have also been demonstrated in the preoptic region of other mammals as the guinea pig (56), the ferret (33), the gerbil (57,104), and the man (63,64).

*The Vomeronasal Pathway*

It is of great interest to note that the medial preoptic region of rodents receives sensitive inputs from the so called vomeronasal pathway [for a review see Segovia and Guillamon (65)]. This pathway has been recently described as a sexually dimorphic network because differences based on sex have been found throughout this chemosensitive pathway (Fig. 5). These include the vomeronasal organ (114), its primary projection [accessory olfactory bulb (115)], the secondary and tertiary projections (116,117). With the exception of the bed nucleus striae terminalis (BNST), the pattern of sexual dimorphism in the whole pathway seems to be homogeneous: males show greater values for volume and number of neurons than females. In the medial anterior division of the BNST the opposite pattern is found (116). According to Segovia and Guillamon (65) the mammalian vomeronasal system might represent a model of how an entirely sexually dimorphic neural network can support and control sexually dimorphic behaviors related to reproduction.



**Fig. 5** Rat vomeronasal pathway. Schematic diagram of brain regions involved in the vomeronasal pathway. Connectivity and morphological features of the system were reviewed by Segovia and Guillamon (65). VNO: vomeronasal organ; AOB: accessory olfactory bulb; BAOT: bed nucleus of the accessory olfactory tract; MeA: medial amygdaloid nucleus; BSTMA: bed nucleus of the stria terminalis, medial division, anterior part; BSTMP: bed nucleus of the stria terminalis, medial division, posterior part; MPA: medial preoptic area; st: stria terminalis. Large arrow indicates the connections of the MPA with lower centers. All nuclei of the system, with the exception of BSTMA are larger in male. The BSTMA is the only which is larger in female. The histogram shows the differences in volume of these nuclei. For each nucleus the larger volume (in male or in female for BSTMA) was conventionally put to 100 [data reviewed by Segovia and Guillamon (65)].

**DIMORPHIC HYPOTHALAMIC STRUCTURES IN HUMANS**

*The Preoptic Region*

Swaab and Fliers (63) described a sexually dimorphic cell group in the preoptic area of the human hypothalamus. They suggested that the volume of this nucleus is twice as large in men as compared to women based on morphometric analysis (Fig. 6B). The nucleus described was located in the medial preoptic area, between the dorsolateral supraoptic nucleus and the rostral pole of the paraventricular nucleus. It was named, according to the existing nomenclature for the preoptic region of the rat, as the sexually dimorphic nucleus of the preoptic region (SDN-POA) and was later identified as the preoptic intermediate nucleus (118). In a further study, Hofman and Swaab (119) detailed to a larger extent the size, shape, and cellular morphology of the SDN. The morphology of the SDN-POA was compared with that of other surrounding hypothalamic regions (the suprachiasmatic nucleus, and the paraventricular nucleus) and these areas were not found to have sexual

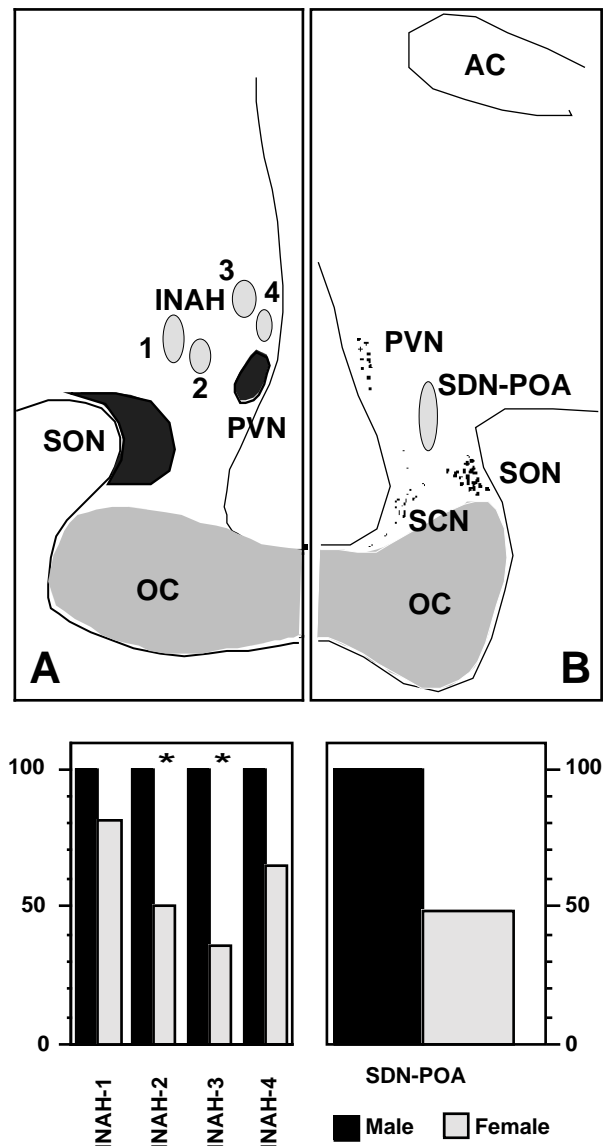
dimorphism. In a subsequent study, Allen et al. (64) performed a quantitative analysis of the volume of 4 cell groups in the preoptic-anterior hypothalamic area and supraoptic nucleus in 22 age-matched human subjects (11 men and 11 women). These authors elected to name the cell groups as the Interstitial Nuclei of the Anterior Hypothalamus (INAH) in numerical order (1 to 4) in a lateral to medial direction (Fig. 6A). Morphometrical measures were done on 60µm-thick sections. No sexually dimorphic characteristics were observed in the INAH-1 area, identified, according to its location with the SDN-POA of Swaab and Fliers (63). However, gender-related differences were found in other two groups. One (INAH-3) was about 3 times larger in male than in female brain. The other cell group (INAH-2) was twice as large in the male brain, but also appeared vary in women relative to circulating hormone levels and was 3.7-fold larger in women of child-bearing age (20-32 years old) than in prepubescent or postmenopausal females. The discrepancies between these two studies may be related to differences in the methods of volumetric quantification, in the thickness of sections, and in the range of ages. According to Tobet and Fox (42) [based also on the report of Le Vay (1,120)], it might be that Swaab and Fliers (63) examined a combination of regions including the INAH-1 and INAH-2 of the classification of Allen et al. (64). In a recent review Swaab et al. (4) noted that since immunocytochemistry was not performed in the study of Allen et al. (64) it is not clear whether their nuclei have to be considered as subdivisions of the paraventricular nucleus or as separate anatomical entities.

Some additional information concerning the course of development and aging are available for the SDN-POA. Swaab and coworkers (4,5,121) described the SDN-POA in a hundred subjects during a period from fetal week 22 up to 93 years of age. According to their data, SDN cell number is equally small in boys and girls at birth, corresponding to about 22% of the cell number found at 2-4 years of age. After the age of 2-4 years (when the cell number peaked), sexual differences were found in the SDN apparently due to a decrease in cell number in women, whereas the cell number in men remains approximately unchanged up to 50 years. It was noted that in men the reduction in cell number in senescence is less dramatic than in women. At each stage of development and aging the SDN volume followed a course similar to that of the SDN cell number.

*The Nucleus of the Striae Terminalis*

A clear sex differences was described by Allen and Gorski (122) in a subdivision of the human BNST. The authors performed a quantitative analysis of the volume of the darkly staining region of the posteromedial BNST (BNST-dspm) on the brains of 26 age-matched male (n=13) and female (n=13) human subjects. They found that the volume of the BNST-dspm was 2.5 times greater in males than in females. This region in humans appears to correspond to an

area of the BNST in laboratory animals that exhibits both volumetric and neurochemical sexual dimorphism, concentrates gonadal steroids, and connects anatomically to several other sexually dimorphic nuclei (65,116).



**Fig. 6.** Sexually dimorphic nuclei of the human hypothalamus. The two schematic drawings show the distribution of nuclei according to Allen et al. (A) or Swaab and Fliers (B). The drawings were rearranged from Le Vay (120) and Swaab and Fliers (63). Differences in volume are reported in the histograms. The male nuclei were conventionally put equal to 100. On the left data from Allen et al. (64) are reported. Stars indicate nuclei with statistically significant sexual dimorphism (INAH-2 and -3). On the right data for SDN-POA are reported [from Swaab and Fliers (63)].

*A neural basis for sexual orientation?*

Data elucidating the mechanisms underlying sexual differentiation of human brain structures has remained elusive due to a variety of reasons. Unlike laboratory animals, hormonal exposure during differentiation may be extremely variable,

due to individual variation. In some clinical conditions, which result in excessive or insufficient hormone exposure, data collected from these individuals have yielded confusing information. An alternative approach has been to attempt to identify regions of the human brain that are sexually dimorphic and subsequently examine those structures in subjects who have been exposed to pathological perinatal steroid hormone levels. At this time, only a few of these studies have been conducted. As previously reported, Allen and coworkers (64) reported changes related to the age and possibly to the levels of gonadal hormones in the female INAH-2 nucleus. In addition, two morphometrical studies comparing heterosexual and homosexual men demonstrated statistically significant differences in two nuclei. Of these studies, Swaab and Hofman (123) found that the volume of the suprachiasmatic nucleus (SCN) in homosexual men (n=10) was 1.7 times larger than that of a reference group of male subjects (n=24) and contained 2.1 times as many cells. The SDN-POA, located in the immediate vicinity of the SCN, exhibited no such differences in volume or cell number. These data hence indicate the selectivity of the enlarged SCN in homosexual men, but do not support the hypothesis that homosexual men have a female hypothalamus. In contrast, Le Vay (120) measured the INAH 1-4 nuclei from three subject groups: women, men who were presumed to be heterosexual, and homosexual men. The INAH-3 was significantly larger in the heterosexual men compared to women and to homosexual men. This finding, in contrast with that of Swaab and Hofman (123) on SDN-POA, indicates that INAH-3 is dimorphic with sexual orientation, at least in men, and suggests that sexual orientation may have a biological substrate. Altered hormone levels may also occur with changing physiological state, for example increasing age is accompanied by decreased sexual activity and deterioration of gonadal function [for review see (6)]. A decrease in testosterone concentration has been observed in human males between 45-65 years, as well as a reduced estradiol and progesterone secretion in postmenopausal females. It is not clear if the decrease in cell number of the human SDN-POA which starts dramatically around the age of 50 in males is a direct consequence of age-related alterations of gonadal functions (119), however the apparent parallelism of the two phenomena and the larger amplitude of them in the human female (in which the decrease of gonadal hormones levels has a more marked course) are suggestive of a strong correlation and of the involvement of this structure in the control of some aspects of sexual behavior.

Reinisch et al. (124) reviewed 19 studies on the behavioral effects of prenatal exposure to hormones administered for the treatment of at-risk human pregnancy. From these studies it appears that prenatal exposure to androgen-based synthetic progestin exerted a masculinizing and/or defeminizing influence on human behavioral development, whereas prenatal exposure to natural progesterone or progesterone-based synthetic progestin had a feminizing and/or demasculinizing influence, particularly among female

subjects. The picture that emerges, even if methodological aspects of the researches are lacking, is generally consistent with the specific literature based on animal experimentation. The prenatal hormonal environment should exert a predisposing influence on adult sexual orientation, although postnatal socialization also makes a very important contribution (125).

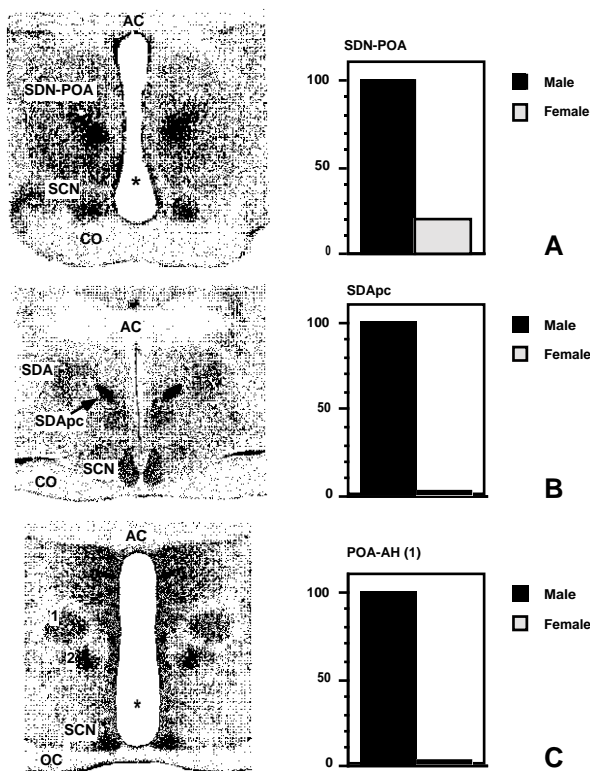
The role of genetics in male sexual orientation was recently investigated (3) in 114 families of homosexual men. Increased rates of same-sex orientation were found in the maternal uncles and male cousins of these subjects, but not in their fathers or paternal relatives, suggesting the possibility of sex-linked transmission in a portion of the population. DNA linkage analysis suggested that the subtelomeric region of the long arm of the sex chromosome (region Xq28) might be involved in determining at least one subtype of male sexual orientation.

In summary, data collected to date on the human brain cannot explain if anatomical and neural differences in sexually dimorphic areas [not only in the hypothalamus, but also in the BNST (122), in the corpus callosum (126-129), in the anterior commissure (130), in the brain weight (131)] relate to the findings of behavioral and functional sexual dimorphism. Moreover, the morphometrical data are controversial, and this is in part due to the obscure nomenclature for some parts of the human brain (such as the preoptic region), and, perhaps to the fact that individual brain differences might be more marked than the sexual differences. The number of subject investigated is always very low, with strong differences in age, and with a poor knowledge of the physiology of the individuals before death. It is hence possible that the reported morphological differences are a direct result of activational effects of gonadal hormones rather than to be the expression of organizational differences. Finally, the genetic bases for these processes remain unclear.

#### **ANIMAL MODELS: SEXUALLY DIMORPHIC STRUCTURES OF PREOPTIC-HYPOTHALAMIC REGION**

In view of the problems in studying human, it is apparent that studies on animal model systems are critical in elucidating the mechanisms involved in behavioral and neurobiological differences between sexes. Considering the wide number of available experimental models (see above), we will restrict our review to those based on preoptic-anterior hypothalamic structures, which are usually considered involved in the control of male sexual behavior.

In mammals, birds, reptiles, and amphibia, studies have demonstrated the presence of sexually dimorphic structures within the POA and the anterior hypothalamus. These structures appear to vary from one species to another, homologies are not easily applicable and they were never fully



**Fig. 7.** Sexually dimorphic structures in the preoptic region of mammals. On the left drawings of the corresponding regions are reported. On the right the sexual difference of the volume is illustrated. The volume of the structure in the male was conventionally put equal to 100.

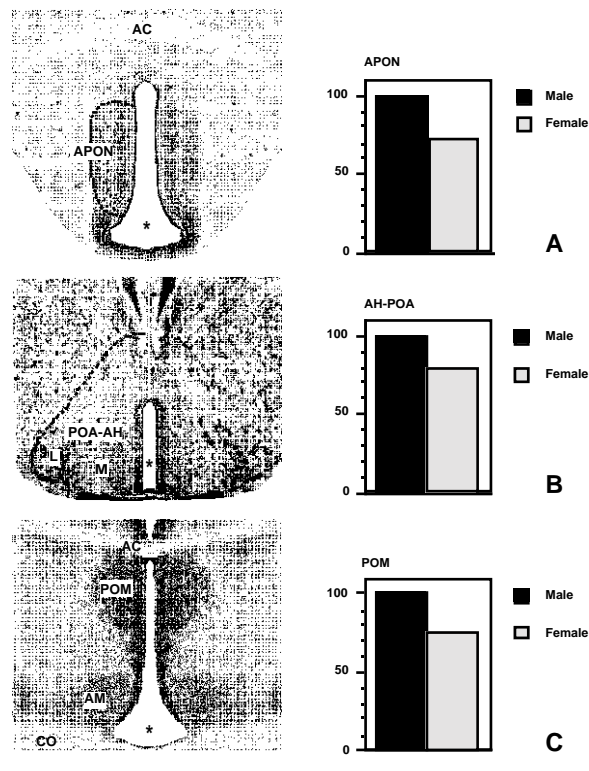
A. Rat. The sexually dimorphic nucleus (SDN-POA) is located within the medial preoptic nucleus. AC: anterior commissure; SCN: suprachiasmatic nucleus; CO: optic chiasma; the star indicates the third ventricle. The drawing is according to the pictures of Gorski et al. (113).

B. Gerbil. The pars compacta of the sexually dimorphic area (SDApc) is indicated by the arrow. This structure is not visible in the female brain. The drawing is according to the pictures of Commins and Yahr (57).

C. Ferret. In the preoptic region-anterior hypothalamus (POA-AH), two nuclei were identified (1: dorsal; 2: ventral). The dorsal nucleus is lacking in the female brain. The drawing is according to the pictures of Tobet et al. (33).

discussed in the literature (1,42,50,112,132). Moreover, anatomical terminology is confusing in the delimitation and identification of structures across the species. This problem is even more pronounced when comparing different vertebrate classes. The preoptic sexually dimorphic structures represent, in fact, the result of the interaction between the hormonal environment and specific neural structures whose extension, functional relevance, and connectivity may vary to a large extent also among closely related species of the same class. A good example of these differences is represented by the vocal control pathways in birds (82).

In the rat, part of the medial preoptic nucleus has a neuronal density significantly larger than in surrounding regions (55,132). The volume of this region which was at first named sexually dimorphic nucleus (SDN, Fig. 7A) is about five times larger in males than females (113). Castration of the male at day



**Fig. 8.** Sexually dimorphic structures in the preoptic region of non-mammalian vertebrates. On the left drawings of the corresponding regions are reported. On the right the sexual difference of the volume is illustrated. The volume of the structure in the male was conventionally put equal to 100. A. Toad. The anterior part of the preoptic nucleus (APON) is significantly larger in male than in female toad (*Bufo japonicus*). The drawing is according to the pictures of Takami et al. (138). The star indicates the third ventricle B. Whiptail lizard. The volume of the anterior hypothalamus-preoptic area (POA-AH) is larger in male lizard (*Cnemidophorus inornatus*). The drawing is according to the pictures of Crews et al. (61). L and M: lateral and medial part of the POA-AH. C. Japanese quail. The volume of the medial preoptic nucleus (POM) is larger in male quail (*Coturnix japonica*). The drawing is according to the pictures of Viglietti-Panzica et al. (58). AM: nucleus anterior medialis hypothalami.

1, postpartum, significantly reduced the SDN volume in the adult; exogenous T given to either genetic female or 1-day castrated male significantly increased nuclear volume in adulthood (133). This appears to be a clear example of organizational effect of the T at the time of birth, as SDN volume during the fetal life is not dimorphic (134). A second sexually dimorphic region is the anteroventral periventricular nucleus (AVPv) which is located, in the rat, bilaterally in the periventricular gray area of the POA. The AVPv of female rats is larger and has a higher density of cells than that of males at adulthood (135). Prenatal or neonatal treatment of female rats with T-propionate induces a significant reduction in the size of AVPv when these rats are examined at adulthood (136,137).

Studies in other mammalian species have yielded interesting data that are somewhat species

specific. Commins and Yahr (57) demonstrated that a more compact part of the sexually dimorphic nucleus (pars compacta) of the gerbil is present in the male but not in the female (Fig. 7B). This region is also steroid sensitive in the adult and disappears in castrated males. It is observed after T treatment of castrated males. This represents an example of both organizational and activational actions of T on the same region.

In the ferret POA-anterior hypothalamus, Tobet and coworkers (33) described a dorsal nucleus that was apparent only in males (Fig. 7C), irrespective of hormonal status (castrated, or supplied with T or progesterone). There was a significant effect of hormonal treatment on cell area with an increase of the size in T-treated males. In this case, the presence of the nucleus appears to be a true sexually dimorphic feature, which is dependent on an organizational action of steroid hormones and with variation in the size of the cells according to the effect of T.

In the toad, the anterior part of the preoptic nucleus [which is not a direct homologue of the medial preoptic nucleus of higher vertebrates (138)] and the amygdala are larger in the male than those in the female (Fig. 8A) (60). However, experimental observations demonstrated that only the amygdala volume is sensitive to the castration during the autumn; while variations of the volume of the preoptic nucleus do not reach the statistic significance (139).

The whiptail lizard (*Cnemidophorus inornatus*) represents an interesting model to study sexually dimorphic dual neural circuits together in the same individual. Crews and coworkers (61) have in fact found that the POA is larger in males than in females (Fig. 8B), whereas the ventromedial hypothalamus is larger in females than in males. The volume and cell size of these structures are also controlled by gonadal hormones in the adulthood (140,141).

Morphological and functional studies conducted during the 80s tested the hypothesis of a link between sexually dimorphic structures and the control of reproduction in several animal models. In numerous mammalian species large lesions of the medial POA profoundly disrupt masculine coital performance (but not motivation). However, lesions that are restricted to sexually dimorphic portions of the medial POA in rat and ferret cause only minor deficits of masculine coital performance [reviewed by Cherry and Baum (142)]. Moreover, at least three factors make difficult to relate structural sex differences in the brain to sexually dimorphic functions. First, sexually dimorphic structures are always found in areas implicated in more than one function. For example, the POA not only regulates male socio-sexual behavior, but also female sexual behavior, gonadotropin release, body temperature, osmolarity of extracellular fluids, and sleep rhythms. Since many of these functions are sexually differentiated, it is hard to determine the relative impact of observed sex differences on each of these functions. Second, the neural connections between sexually dimorphic areas are poorly documented. It is, therefore, often unclear which other brain areas might be influenced by a given sex

difference. Third, many of the reported structural sex differences are not influenced by sex steroids in adulthood, although many of the sexually differentiated functions are so influenced. This makes it more difficult to identify which cellular systems in a sexually dimorphic area might contribute to functional sex differences.

### **JAPANESE QUAIL: A MODEL FOR THE STUDY OF SEXUALLY DIMORPHIC BEHAVIOR AND MORPHOLOGY.**

Recent studies have taken advantage of avian model system for study of neural mechanisms that underlay sexually dimorphic reproductive behavior. However the majority of this research has dealt with the song control system and, as discussed by Arnold (143), and Arnold and Schlinger (41), the pattern of sexual differentiation of this system does not fit the classically defined pattern of sexual differentiation of avian behaviors and secondary sexual characteristics. In recent years, the Japanese quail (*Coturnix japonica*) became one of the most well studied models for investigation of these relationships (144,145). Several dimorphic characteristics have been described in the quail including: color or plumage, structure of calls, and sensitivity to sex steroids [for a review, see Ottinger (146)]. In contrast to mammals, the female quail does not become masculinized by steroid exposure during the perinatal period. Reproductive condition is regulated by photoperiod and the mechanisms involved have been studied extensively (147-157).

A number of endocrine and behavioral studies have demonstrated that courtship and mating behavior are T dependent. Castrated male quail do not show any attempt to copulate, whereas courtship and mating behaviors are restored by subcutaneous implants of T (158). During sexual maturation, there is a highly correlated relationship of T with testicular development and ontogeny of courtship and mating behavior (159-161). Later research provided evidence that the POA is the major target for the regulatory effects of gonadal steroids (162,163). Further, it appears that T serves as a "prohormone" in the CNS and is first metabolized to estradiol by the enzyme aromatase, in order to exert behavioral effects (164,165) [for a review, see Adkins-Regan (166)]. Additionally, T has been found to induce a significant increase in aromatase activity in the male preoptic region, with no effect in the female (101). Because of these data, our research has concentrated investigations on the anatomy and neurochemistry of the anterior hypothalamic-preoptic region of quail, in normal as well as in hormonally manipulated birds. We will review these topics as well as age-related changes in these systems.

*The medial preoptic nucleus of the Japanese quail*

Morphometrical investigations demonstrated that within the anterior hypothalamic-preoptic region, only the medial preoptic nucleus (POM) is sexually dimorphic in the adult (Fig. 8C). The volume of the POM is approximately 1/3 larger in adult male than in female quail (58,167). This dimorphism is affected in adulthood by circulating concentrations of T and, as such, represents an activational effect of this hormone. Increases in POM volume is found in T-treated ovariectomized females, and the volume of the nucleus decreases in castrated males (34,35,59) or in intact males kept in short-day conditions (35). This nucleus is characterized by a complex cytoarchitecture and the presence of several peptidergic cells and/or fibres [for review, see Panzica et al. (168)]. Immunohistochemical studies demonstrate a large population of aromatase-immunoreactive neurons specifically belonging to the nucleus (169,170), as well as the presence of estrogen (27) and androgen receptors (88). Cytoarchitectural and morphometrical studies provide evidence for the existence of two neuronal populations, located in the medial and in the dorso-lateral portions of the POM and distinguished by their size. These two populations are characterized by their different sensitivities to T in adulthood. The medial population appears smaller and relatively insensitive to T, whereas the dorso-lateral population (only in males) changes in size in correlation to the levels of T (35,171). Ultrastructural studies suggest that T is influencing specific cellular compartments involved in the synthesis and processing of proteins (172). Experimental studies performed by stereotaxic implantation of crystalline T in castrated males, or by electrolytic lesions of intact males have shown that the POM (chiefly its dorso-lateral aspect) is a critical site of steroid action in the control of copulatory behavior (173,174). Further, the sensitivity to T of the dorso-lateral neuronal population in adult males suggests that these cells were irreversibly affected by an organizational effect of T. Evidence for this hypothesis was provided from results of administration of estradiol benzoate *in ovo* before the 12 day of incubation. It is well known that this treatment inhibits both male copulatory behavior, as well as behavior induced by T in gonadectomized male (175). After this treatment, the dorso-lateral population of the POM contained significantly smaller neurons which were insensitive to T (176). Further morphometrical and behavioral studies (177) illustrated that changes in the size of the dorso-lateral population, as well as the copulatory behavior, were stimulated by the simultaneous presence of both principal metabolites of T (17 $\beta$ -estradiol and 5 $\alpha$ -DHT). These studies reveal that the POM is a critical element in the regulation of reproductive behavior and that there is an apparent action of T and of its metabolites (144). Recent morphometric studies (178,179) revealed that the aromatase immunoreactive neurons, largely in the lateral part of the POM, are sensitive to the levels of T. They also decrease in number and size in castrated males confirming biochemical data on the induction of this enzyme by the T (101).

The POM is characterized by a heterogeneity of neurochemical markers. Immunohistochemical studies have not only demonstrated the presence of receptors for androgens, estrogens and the enzyme aromatase, but also the  $\alpha_2$ -adrenergic and muscarinic receptors, the enzyme NO-synthase, and neuropeptidergic cells containing neurotensin, NPY, substance P and LHRH. Moreover catecholamine-, serotonin-, vasotocin-, substance P-, CRF-, NPY-, VIP- and NO-containing fibres demonstrated different patterns of distribution, contacting several different cell subgroups within the POM [for review see Panzica et al. (168)]. Recent studies involving the use of the Dil on fixed brains demonstrated that the POM is linked to several telencephalic, diencephalic, and brain stem regions (180,181). In particular, the nucleus is connected with the following areas: 1) septal region, in which the largest population of LHRH neurons reside (182), 2) paraventricular nucleus, which is the main center controlling the neurovegetative activities (183,184), and 3) central mesencephalic grey, which is the main control center for the copulatory activities in mammals (185-187). Immunohistochemical studies have shown some afferent systems are also controlled by T. In fact, a sexually dimorphic distribution of vasotocinergic fibres in the septo-preoptic region has been described, which is sensitive to T only in the male quail (188,189).

*Reproductive Aging and the quail POM*

Many species, including quail (as well as rat and primates) have two phases in the deterioration of reproductive status [for review, see Ottinger (15)]. The first is evidenced by declining incidence of reproductive behavior, often correlated with reduced fertility, but without changes in plasma T. The next phase includes hormonal and gonadal signs of reproductive dysfunction leading to reproductive senescence. Aging male quail exhibit elevated plasma levels of estradiol during middle age, which declines thereafter. As previously discussed, estradiol is the active metabolite of T in the CNS, and is formed by the action of the enzyme aromatase, it is very interesting to note that reproductive behavior can be restimulated in senescent male quail by exogenous T. Due to the fact that POM and mainly the aromatase cells of this nucleus are the main target for the action of steroid hormones in the preoptic region (178) and are also the center controlling male copulatory behavior, it was of interest to study the aromatase system in old male quail. A recent study (190) investigated the immunocytochemical distribution of aromatase containing cells in young active male quail compared to old inactive or old active males. The number of ARO cells in the preoptic area was sharply reduced with little change in staining for estradiol receptors. This reduction in the immunoreactive aromatase staining was due to a significant loss of number of cells staining positive

in the medial part of the POM (191). Further, aged sexually active males retained relatively more and larger aromatase positive cells than aged inactive males. In other studies (178), it was found, on the contrary, that young castrate males showed a greater loss of ARO cells in the lateral as compared to the medial portions of the nucleus. Two conclusions can be drawn from these experiments: a) the aromatase system is specifically influenced by the reduction of circulating T both in the young and in the old animal, and loss of part of this neuronal system resulted in a loss of the sexual performances; b) the specific mechanisms of the effect of hormone decline on the aromatase system may vary in the young and in the old male quail (191). Moreover the increase in cell size observed in old sexually active males can be considered as a compensatory effect to minimize the effect of a deficiency production of aromatase linked to the reduction in number of these elements (191).

In conclusion, the medial preoptic nucleus of the Japanese quail represents an excellent model to study the functional organization of neuronal circuits controlling reproductive behaviors. Within the POM, there is topographic and functional segregation, with the dorso-lateral portion implicated in the control of copulatory behavior. The POM is exquisitely sensitive to T and its metabolite, estradiol. This is reflected by changes of the size, the intensity of staining, as well as ultrastructural organization. These modifications may mirror increased synthesis of the enzyme aromatase, which produces estradiol. The sensitivity to T is organized during the embryonic development, with reversal only by administration of the antagonist hormone during a critical period of development. The behavioral and morphological effects of T are due to its main metabolites (estradiol and 5\_DHT) which can stimulate the surrounding estrogen- or androgen-receptor bearing neurons of the nucleus. It is important to note that a relatively small subset of aromatase-containing neurons are also immunoreactive for estrogen-receptors (192). The POM neurons are connected with important centers regulating neurovegetative activities, as well as the secretion of gonadotropin-releasing hormone and control of motor activities linked to copulation. Moreover, some of the afferent pathways can be, in turn, controlled by T, and contribute to regulate the activity of the POM neurons in a steroid-dependent way.

The complex of these observations together with those collected from other animal models emphasizes that factors regulating sexual behavior (hormones, sensitive cells, neurotransmitters, neuropeptides) do not act independently, but in an integrated manner. In summary, several points may be reiterated. First, circulating gonadal hormones act on sensitive target areas directly as well as through their metabolites which were generated by the neurons containing the appropriate enzymes. Second, neurons which are the target for gonadal hormones vary their metabolic activities resulting in varied levels of synthesis of many products including enzymes implicated in neurotransmitter production, precursors for

neuropeptides, and receptors. This is reflected by the increase of cell size and by the reorganization of the ultrastructure. Third, part of the afferent pathways can be also regulated by gonadal hormones through an influence on their centers of origin, or through a direct membrane effect influencing the rate of secretion of the neurotransmitter or neuropeptide.

All these findings point to the critical importance of studies like those briefly summarized here, many of which have been accomplished through a multidisciplinary approach (behavioral, neuroendocrine, morphological, physiological). This is the best strategy to improve our fundamental understanding of the interactions taking place in the brain.

#### ACKNOWLEDGEMENTS

These studies were supported by grants from CNR (93.00372, 94.02462), MURST (60% and 40%), USDA (88-37242-2913), NRI (92-37203-7742), NATO (CRG.921267), EC (CT94-0472), and ESF (RG 14/1992).

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