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Sexual Differentiation of the Vertebrate Brain: Principles and Mechanisms

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A wide variety of sexual dimorphisms, structural differences between the sexes, have been described in the brains of many vertebrate species, including humans. In animal models of neural sexual dimorphism, gonadal steroid hormones, specifically androgens, play a crucial role in engendering these differences by masculinizing the nervous system of males. Usually, the androgen must act early in life, often during the fetal period to masculinize the nervous system and behavior. However, there are a few examples of androgen, in adulthood, masculinizing both the structure of the nervous system and behavior. In the modal pattern, androgens are required both during development and adulthood to fully masculinize brain structure and behavior. In rodent models of neural sexual dimorphism, it is often the aromatized metabolites of androgen, i.e., estrogens, which interact with estrogen receptors to masculinize the brain, but there is little evidence that aromatized metabolites of androgen play this role in primates, including humans. There are other animal models where androgens themselves masculinize the nervous system through interaction with androgen receptors. In the course of masculinizing the nervous system, steroids can affect a wide variety of cellular mechanisms, including neurogenesis, cell death, cell migration, synapse formation, synapse elimination, and cell differentiation. In animal models, there are no known examples where only a single neural center displays sexual dimorphism. Rather, each case of sexual dimorphism seems to be part of a distributed network of sexually dimorphic neuronal populations which normally interact with each other. Finally, there is ample evidence of sexual dimorphism in the human brain, as sex differences in behavior would require, but there has not yet been any definitive proof that steroids acting early in development directly masculinize the human brain. Key Words: Sexual dimorphism, sex differences, birdsong, vomeronasal system, hippocampus.

INTRODUCTION

Thirty years ago there was already widespread agreement that there must be sexual dimorphism—structural differences between the sexes—in the brain. But there is little indication that anyone was searching for these neural sexual dimorphisms. Perhaps it was assumed that the differences would be so subtle that our neuroanatomical techniques would be unable to detect them. We have learned since that either our neuroanatomical techniques are not so crude or

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sexual dimorphism in the vertebrate nervous system is not so subtle. Sexual dimorphism is found throughout the nervous system of vertebrates. So many sexual dimorphisms have been found in so many regions of the brain that a simple listing would be rather dull. So we will instead review some instances of sexual dimorphism in the nervous system that have been particularly fruitful. Examination of these selected models will illustrate several themes: the crucial role played by steroid hormones in directing sexual dimorphism; the variety of cellular mechanisms that can be altered by steroid hormones; the role of the periphery in directing the nervous system; the still largely untested role of experience; and, finally, the relevance of these findings for understanding sex differences in human behavior.

Sex Differences in Behavior Foreshadowed Sexual Dimorphism in the Brain

In the wake of Alfred Jost's pioneering work explicating the role of testicular hormones in organizing the internal and external genitalia of mammals, Phoenix, Goy, Gerall, and Young (157) suggested an extension of these ideas to the brain. Based on inferences from the behavior of guinea pigs, they proposed that testicular steroids could permanently alter the developing nervous system to make it more likely to display masculine behaviors, and less likely to display feminine behaviors, in adulthood. This long-lasting "organizational" effect of steroids stood in contrast to the well established "activational" effects of steroids on behavior, wherein steroid treatment of adult animals caused a temporary change in the probability that the animal would show some behavior. An example of an activational effect is the induction of a few hours of sexual receptivity in female rodents treated with estrogen followed by progesterone. In contrast, exposure of female rodents to androgens *in utero* could permanently reduce their receptivity in adulthood (157), and the permanence of such effects seemed more like an organization of the nervous system rather than a mere activation. While there are examples of phenomena that do not fit this simple dichotomy (9), there is no denying the heuristic value of the idea or that steroid hormones can indeed permanently alter the developing brain in a fundamental, organizational, manner. Perhaps the most important contribution of the organizational hypothesis was to draw attention to the question of why males and females behave differently. After this landmark paper (157) the question was refined to, "what is different about males and females that causes them to behave differently?" Once this question was posed, the most obvious potential answer was that the brains of males and females were structurally different. The organizational hypothesis also suggested a mechanism by which male and female brains might become different during development, i.e., by the intervention of steroidal hormones secreted from the gonads. Indeed, sexual dimorphism in the nervous system has been described at virtually every anatomical level—molecular, ultrastructural, cellular, and neural systems levels. Furthermore, the structural differences leading to sex differences in behavior are

spread throughout the nervous system rather than concentrated in a single structure or system.

An important aspect of androgen's organization of the brain in rodents is that many androgens can be converted, in a single chemical reaction, into an estrogen. This reaction is a chemical aromatization which is controlled by the enzyme aromatase (141). Thus it is often the interaction of androgen's estrogenic metabolite with estrogen receptors, rather than androgens interacting with androgen receptors, that initiates the masculinization of the rodent brain. These observations led to the development of the "aromatization hypothesis" of brain masculinization. As we will see, aromatization also seems to play a role in the masculinization of the songbird brain. However, it may not be important for masculinization of the brain in primates.

A SELECTIVE INVENTORY OF SEXUAL DIMORPHISM IN THE VERTEBRATE NERVOUS SYSTEM

We will describe several models of sexual dimorphism and briefly review what is known about their function and, for each system, the role of steroid hormones in the development and/or maintenance of the dimorphism. Then we will discuss several general principles that can be derived from these models before taking up humans.

The Song System of Birds

No review of brain sexual dimorphism would be complete without mention of the songbird system, but because there have been several major reviews of this system recently (7, 8, 19), we will touch only briefly on songbirds and concentrate on discussions of other sexually dimorphic CNS systems. The widely accepted view that the brains of males and females must be structurally different was confirmed by Raisman and Field's (161) seminal report that female rats had a greater proportion of certain synaptic types in the preoptic area (POA) than did males. Despite these reports of ultrastructural sexual dimorphism in the rat brain, it came as something of a surprise when Nottebohm and Arnold (147) reported that the song control nuclei in the brain of zebra finches and canaries were five to six times larger in volume in males (which sing frequently) than in females (which sing very little). This large sexual dimorphism could be detected in brain sections without resorting to a microscope. An important feature of the birdsong system is that the sexual dimorphism is quite clearly related to sex differences in a complex, learned behavior. The various brain regions involved in song production are most readily identified by their relationship to the vocal organ or syrinx. Motoneurons of the XII nucleus control the muscles of the syrinx to modulate sound frequency, etc., and these motoneurons in turn receive considerable synaptic input from the Robustus Archistriatum or RA (148). RA receives synaptic input

from an evolutionarily, literally, and nominally higher nucleus, the higher vocal center or HVC (previously called the hyperstriatum ventrale caudale nucleus). All three brain regions (XII n., RA, HVC) are volumetrically larger in males than in females in both zebra finches and canaries. Among bird species in which both females and males sing, these brain regions are sexually monomorphic, or nearly so (27, 73).

The Role of Steroids in the Birdsong Brain

In zebra finches it appears that the aromatization of testicular androgens into estrogens causes the developing brain to be masculinized (84). Female zebra finches treated early in life with estrogens have masculinized brain nuclei and will sing as adults (but only if they are also given an "activational" dose of androgen in adulthood). So the birdsong nuclei of zebra finches behave in good accordance with both the organizational hypothesis and the aromatization hypothesis. Early steroid treatment seems to affect the number of neurons in zebra finch brain regions (84), while adult steroids increase the dendritic extent of the neurons (53, 54), so there are clear morphological indicators of both the organizational and activational effects of hormones. Both activational and organizational influences are required for the female zebra finch to sing.

Canaries are somewhat different from zebra finches because the canary birdsong nuclei remain plastic into adulthood. Thus androgen treatment of even an adult female canary will result in structural alterations of her birdsong brain nuclei and she will sing (75). There is little point in arguing whether canaries refute the organizational hypothesis or simply amend the hypothesis to include the possibility of marked effects in adulthood. The important point illustrated by canaries is that sex differences in adult behavior can be caused by sex differences in hormone exposure during adulthood, not just during development, and that these effects can be detected in brain structure. No such demonstration has yet been shown for the volume of any mammalian brain nucleus but, as was pointed out some years ago (9), there is little evidence that anyone asks such questions in a mammal. There are, however, several reports of adult steroid treatment altering the size of individual neurons, as we discuss below.

One troublesome aspect of the effects of steroids on birdsong neural centers is that although steroid treatment of females can effectively masculinize neural centers and behavior, the reciprocal manipulation, depriving males of steroid, has not successfully prevented masculinization of the brain. Neither surgical castration, nor anti-estrogen treatment, nor anti-aromatization therapy has prevented male zebra finches from developing a masculine brain and the singing that accompanies it (7). It is always possible that these manipulations have failed to sufficiently affect circulating steroid levels. However, there is also serious speculation that the brains of male songbirds may have an alternative, steroid-independent means of developing in a masculine fashion, perhaps via a direct genetic mechanism (8). We can expect exciting new perspectives to arise

should this alternative mechanism be demonstrated, as workers would then reexamine mammalian systems for evidence of subtle genetic, nonhormonally mediated components of sexual differentiation.

The Song System of Frogs

African clawed frogs, *Xenopus laevis*, also use vocalizations for social communication. Although both male and female frogs are capable of vocalizations, courtship singing is a behavior specific to males. Male frogs produce courtship songs to attract and excite females (173). The neuromuscular system underlying frog singing behavior is composed of motoneurons of the cranial nerve nucleus IX–X, located in a slender column in the caudal medulla. These motoneurons send axons through cranial nerves IX–X to the larynx, where the vocalizations are produced. The larynx is composed of cartilage and the laryngeal dilator muscle. When this muscle contracts, discs resembling bell-shaped clappers are pulled apart and a click sound is produced (108). Trills of clicks make up the frog songs. The courtship call consists of fast and slow trills chained together in repetitive bouts.

The neuromuscular system underlying frog song is sexually dimorphic. Adult males have two large laryngeal dilator muscles made up of about 16,000 fast-twitch fibers each, whereas adult female muscles are much smaller (about 2,000 fibers) and are composed mostly of slow-twitch fibers (175, 176). This difference in muscle morphology partially accounts for the sexual dimorphism in frog song: female laryngeal muscles are simply not capable of contracting rapidly enough to produce the fast trills of courtship songs (203). Functional differences at adult neuromuscular junctions in laryngeal muscles also contribute to differences in vocal behavior. Adult females release greater amounts of transmitter (they have a higher quantal content) than either adult males or juvenile frogs of either sex (205). As a result, male muscles are able to contract and relax more quickly and are thus able to produce rapid trills.

The neurons that constitute the courtship song neural circuit are also sexually dimorphic. The motoneurons of cranial nerve nucleus IX–X that innervate the laryngeal muscles are larger and more numerous in males (87, 193). As expected, there are also a larger number of axons in the laryngeal nerve of male frogs than of females (109). The dendrites of these neurons are also longer in males (110, 111). As part of the "calling circuit" the laryngeal motoneurons of males send recurrent collaterals to neurons of the pretrigeminal nucleus of the dorsal tegmental area of the medulla (108). These collaterals are absent or greatly reduced in females.

Like other amphibians, *X. laevis* begins development as a tadpole for several weeks before metamorphosis into the immature frog. Tadpoles are not sexually differentiated and their gonads remain undeveloped. The gonads remain undifferentiated until late metamorphosis (108). Various sexually dimorphic aspects of the song system develop at different times. The laryngeal muscles remain monomorphic and feminine throughout metamorphosis, and then become sexu-

ally dimorphic. During the 6 months following metamorphosis, male laryngeal muscles increase their mass and fiber number while in females there is little or no increase in fiber number or size. Male muscle fiber type becomes predominately fast-twitch, whereas female muscle fibers become increasingly slow-twitch (204).

In contrast to muscle, the number of axons in the laryngeal nerve begins to be sexually dimorphic earlier, before metamorphosis is complete (109). The adult number of axons is achieved by a sexually differentiated pattern of axonal addition and loss. In males, the number of laryngeal axons increases dramatically between tadpole stages 56 and 62, whereas in females this increase does not occur. Between tadpole stages 62 and adulthood, a period of axonal loss ensues: overall axon number decreases by 47% in males and by 64% in females.

The Role of Steroids in the Frog Laryngeal System

The greater secretion of androgens by males has been shown to mediate most sexual dimorphisms in the frog song system. Throughout metamorphosis, plasma androgen concentrations are low, and male and female levels are similar (~30 pg/mL). During the next 6 months, androgen concentrations increase in males to levels nine times that of female frogs (108). In contrast, estrogen levels are higher in adult females than in males. Androgen receptors are present in both the laryngeal muscles and their motoneurons. In *X. laevis,* there are two isoforms of the androgen receptor. Both isoforms are expressed in larynx (63, 64), whereas only one is expressed in the CNS (153). The ontogenetic pattern of expression differs for motoneuron and muscle. The developing larynx expresses androgen receptor mRNA from very early tadpole stages (35). In male muscle, androgen receptor expression increases at the end of metamorphosis to a peak just after metamorphosis that remains high for the next 6 months (the period of rapid muscle growth) and then gradually declines to adult levels. Female muscles also express high levels of androgen receptor just after metamorphosis, but expression gradually declines to levels half that of adult male muscles. The adult laryngeal motoneurons also express androgen receptor message in a sexually dimorphic manner; more cells express androgen receptor transcripts in male than in female frogs. Motoneuron androgen receptor expression starts earlier than in muscle, and the adult pattern is established by 2 weeks prior to the start of metamorphosis. The expression of androgen receptor message in these motoneurons is upregulated by exposure to androgen (153).

Although exposure to androgen drives the sexual dimorphisms of both laryngeal muscle and axons, the timing of sexual differentiation differs in the two sites, suggesting that the periods of sensitivity or threshold levels of androgen exposure differ for these two components of the frog song neuromusculature. Postmetamorphic androgen secretion induces marked proliferation and differentiation of laryngeal muscle and cartilage precursors. This androgen exposure is required for masculinization of the larynx. Prepubertal castration

or anti-androgen treatment results in a larynx that is feminine in both cell number and type, whereas administration of exogenous androgens to sexually undifferentiated males or females results in a marked proliferation and differentiation of laryngeal tissues (175, 204). Although androgen receptors are present from early tadpole stages, androgen responsiveness of the larynx does not begin until just prior to metamorphic climax (35). In adulthood, prolonged exposure to androgens can completely masculinize muscles in females whereas shorter exposures do not (204). The results of long-term exposure in females may be permanent: effects are still evident as long as 8 months after testosterone removal. Thus early, organizational effects are not required for masculinization of the female muscles.

The motoneurons innervating the vocal organ are sensitive to androgen earlier in development than laryngeal muscle, which may explain why the motoneurons become sexually dimorphic earlier. Treatment of male tadpoles with anti-androgen results in female-like numbers of axons, whereas anti-androgen treatment of female tadpoles has no effect (169). As expected, when female tadpoles were treated with dihydrotestosterone, axon numbers were increased to male-like values. It has been suggested (169) that laryngeal motoneurons are sensitive to lower concentrations of androgens than are the laryngeal muscles and thus respond to androgen secretion during late tadpole stages at concentrations that are subthreshold for laryngeal development. Thus, laryngeal nerve axons respond to androgens and become sexually dimorphic earlier in development than do the laryngeal muscles.

However, the female laryngeal system remains sensitive to androgens into adulthood. Implantation of female frogs with testes after metamorphosis or in adulthood resulted in increased laryngeal axon numbers and laryngeal muscle fiber numbers to levels equivalent to those of intact males (212). Interestingly, treatment with exogenous androgen had no effect on axon number, suggesting that testicular implants may provide other signals in addition to androgen that are required for masculinization of this system.

Given that sex differences in both laryngeal muscle and motoneurons result from exposure to androgens, it is interesting that sex differences at the neuromuscular junctions of adult laryngeal muscles result from female exposure to estrogens. When juveniles are gonadectomized and treated with estrogen, quantal content values increase significantly (205). In contrast, androgen treatment decreases quantal content in juvenile females but not males.

Either during development or in adulthood, other hormones may modulate the effects of steroid hormones by increasing or decreasing the sensitivity of cells, or by otherwise affecting the responses they make. During the development of *Xenopus*, for example, the hormone of amphibian metamorphosis, thyroxine, is essential for the development of the sexual dimorphisms of the song system. Thyroxine is a steroid-like hormone that is required for and drives metamorphosis. Exposure to thyroxine is required for the development of androgen sensitivity by the larynx: without thyroxine exposure, the muscles are unable to respond to androgen (168). Precocious administration of thyroxine resulted in an early development of sensitivity to androgen by the larynx

(35). However, exposure to thyroxine is not required for androgen receptor expression. Even though androgen receptors are clearly present, without prior exposure to thyroxine the growth and differentiation of laryngeal muscles normally induced by androgen does not occur. A lack of exposure to thyroxine also blocks the laryngeal nerve axons from becoming sexually dimorphic. Although thyroxine is required for the development of sexual dimorphisms of the song system, it is not required for the sexual differentiation of the gonads (168).

Thus, although the sexual dimorphism of the *Xenopus* song system is driven primarily by exposure to androgen just prior to and after metamorphosis in males, other hormones are also involved. Previous exposure to thyroxine is critical for androgen receptor effectiveness and thus for the development of sexual dimorphisms of the song system. Also, in female frogs, exposure to estrogens further feminizes the song system. So the process of sexual differentiation in this system, and likely many others, is more complicated than just the organizational and activational effects of steroid hormones. Interactions between hormones, duration and amplitude of exposure, as well as timing during development are all critical parameters that determine the response of the nervous system and the ultimate state of sexual differentiation.

The Spinal Nucleus of the Bulbocavernosus

A sexually dimorphic neuromuscular system in mammals seems to share many of the characteristics of the sexually dimorphic laryngeal neuromuscular system of frogs. A group of motoneurons in the lower lumbar spinal cord innervate striated bulbocavernosus (BC) and levator ani (LA) muscles which are attached to the penis. The motoneurons form the spinal nucleus of the bulbocavernosus (SNB) in the dorsomedial portion of the ventral horn in lumbar segments 5 and 6 in rats. Not surprisingly, male rats have at least three times as many SNB motoneurons as do females and the neurons are only half as large in females as in males (23). We know a great deal about how this sexual dimorphism comes about, mostly because such neuromuscular systems are so much easier to study than brain systems. Both male and female rats have bulbocavernosus muscles before birth and have SNB cells which synapse upon those muscles (163). However, the muscles and motoneurons normally degenerate shortly after birth in females (146), thus establishing the adult sex differences in number of SNB motoneurons and target muscle fibers.

The Role of Steroids in the SNB System

Perhaps the most compelling evidence that steroids direct masculinization of the SNB system is the feminine state of the SNB in genetically male (XY) rats with a defective structural gene for the androgen receptor, so-called Tfm (testicular feminization mutation) rats (24). Affected Tfm males have testes

and androgen, but their defective androgen receptors are insensitive to the steroid. Thus they develop a feminine phenotype including no BC muscles and fewer and smaller SNB motoneurons than in normal males. As estrogen receptors and aromatase are normal in affected Tfm rats, the feminine state of the SNB in these animals also indicates that estrogen receptors play only a minor role, if any. The effects of various perinatal steroid manipulations on the SNB system of male and female rats conform to this conclusion (25, 26): androgen treatment (but not estrogen treatment) of newborn females results in more SNB motoneurons and more and larger BC muscle fibers, while androgen deprivation of perinatal males results in a feminine SNB system in adulthood. Early estrogens may serve to further masculinize the size of SNB cells, since neonatal estrogen treatment of female rats results in larger SNB cells in adulthood. Supplementation of the neonatal estrogen with androgen increases adult SNB size even more (21).

Perinatal androgen treatment of female rats prevents the developmental death of the SNB system (26). Thus the SNB offers a clear picture of one way in which hormones can "organize" a neural system—prevent ontogenetic death (apoptosis). Because the hallmark of developing vertebrate nervous systems is cell–cell interaction, preventing the demise of one cell group may well affect the fate of others, and in the case of the SNB this means sparing the muscles leads to a sparing of the motoneurons.

How does early androgen masculinize the SNB system? Primarily by sparing the target muscles BC and LA, which secondarily spare SNB motoneurons. The clearest demonstration of this indirect action upon SNB motoneurons is from examination of rats which are mosaic for the Tfm androgen sensitivity. In such animals, SNB motoneurons utilizing the defective androgen receptor gene (*Tfm*) are just as likely to be spared from apoptosis as are SNB motoneurons utilizing a wild-type androgen receptor gene (71). Indeed, both androgen receptor immunoreactivity (105) and steroid autoradiography (102) indicate that SNB motoneurons do not express androgen receptor until after the period of apoptosis is complete.

What does androgen do to the BC/LA target muscles to maintain the SNB system? One emerging story suggests that androgens regulate neurotrophic factors and/or sensitivity to neurotrophic factors to maintain the muscles and therefore their motoneurons. Treating newborn female rats with ciliary neurotrophic factor (CNTF) can prevent involution of both the BC/LA target muscles and the SNB motoneurons (69). But apparently endogenous CNTF does not play a role in SNB development, since CNTF knockout mice have a normal-appearing SNB (70). So it is possible that exogenous CNTF treatment maintains the SNB system by activating some system normally stimulated by a CNTF-like ligand. Support for this conjecture is found upon examination of CNTF-receptor α knockout mice—newborn males have no more SNB motoneurons than their sisters, yet the muscles are dimorphic (70). What endogenous ligand normally binds the CNTF-receptor α to maintain SNB motoneurons, where the endogenous factor is produced and how androgen regulates its synthesis or release remains unknown. Interestingly, the CNTF receptor knock-

out male mouse seems to have normal BC/LA muscles, so androgen apparently activates some other, non-CNTF-receptor-mediated, system to spare the muscles. There is also a concomitant sparing of dorsal root ganglion cells (14) and although it remains possible that androgen acts directly upon these cells to spare them, it seems more likely that they, too, are maintained as a secondary consequence of the maintenance of the muscles.

For the SNB system there are also clear morphological signatures of the activational influence of steroids in adults—when males are castrated, the size of SNB somata and nuclei shrink (24) and the dendritic trees shrink (e.g., 165), and all of these effects are reversed by adult androgen treatment. Again this simple system presents a more tractable problem, i.e., where does androgen act to initiate these changes? Androgen acts directly upon the BC/LA muscles to exert its anabolic effect, as demonstrated by the finding that local implants of androgen affected the size of nearby muscle fibers more than fibers distal to the implant (164), and supported by the classic finding that androgen increases myoblast mitosis *in vitro* (160). Androgen can also affect motoneuronal dendrites by acting upon the target muscles, since local androgen treatment of the muscles on one side of an animal results in greater dendritic branching of the motoneurons innervating that side compared to contralateral motoneurons in the same animal (165). These findings further complicate the picture of how steroid hormones work to influence sexual dimorphism: steroids acting on one cell population may cause them to affect another population. Thus the morphology of cells which do not themselves express steroid receptors can still be indirectly affected by steroids. While systemic androgen treatment augments dendritic extent in every direction, androgen treatment at the target muscle increases dendrites only in the dorsal and contralateral fields, not the ipsilateral field. Thus androgen may act elsewhere to augment ipsilateral dendrites of SNB motoneurons.

So does androgen induce activational influences on the SNB system solely through action on the target muscle? Not according to analysis of mosaic rats. These animals have some SNB motoneurons which express a defective copy of the gene for the androgen receptor (*Tfm*) while other SNB motoneurons (within the same animal) express the normal, wild-type androgen receptor. When such mosaics are treated with androgen for a month, it is only the wild-type SNB motoneurons which display an expanded somata; the androgen-insensitive SNB motoneurons show no such response (71, 213). Since only motoneurons with functional androgen receptor increase somata size, androgen must act directly upon the motoneurons to exert this effect.

The SNB system has also yielded examples of how experience might interact with steroids to alter neural systems and behavior. Celia Moore and colleagues found that rat dams can distinguish their newborn sons from daughters, because they lick the anogenital regions of their male pups more often than that of their female pups. Injecting female pups with androgens attracts more licking from the dam, so probably the dam detects androgenic metabolites in the urine. Dams made anosmic (incapable of distinguishing odors) licked all pups less, and the sons of such anosmic pups possessed fewer SNB motoneu-

rons in adulthood (138). Presumably the sensory stimulation of the anogenital region by the dam contributes to the survival of the motoneurons. Thus it appears that one of the roles of androgen in newborn male pups is to attract more anogenital grooming from the dam to preserve more SNB cells.

Experience in adulthood can also affect SNB structure. Male rats were castrated as adults and given low systemic doses of testosterone—lower than normal circulating levels yet still sufficient to maintain copulatory behavior. Then some of the males were given extensive copulatory experience for a month (by being provided with ovariectomized female cagemates in constant behavioral estrus) while other males had no such experience (being provided with ovariectomized female cagemates that were unreceptive). The somata, nuclei and target muscles of SNB motoneurons from the copulating animals were significantly smaller than those of the noncopulating rats (22). It is interesting that copulatory experience has an effect on SNB neuronal size that is the opposite of androgen, which enlarges SNB motoneurons. Since copulatory experience in males normally increases secretion of androgen, the two may normally act to counterbalance SNB morphology, maintaining the SNB system at a particular state, whether or not the male has copulated recently. It is important to remember that even in this relatively simple model system, external cues can also influence the development and maintenance of the nervous system by providing experience to the nervous system. It suggests that sexual differentiation of more complicated neural structures in the brain, especially in more complicated, highly social species such as humans, may rely even further on experience, in either development and/or adulthood.

The Sexually Dimorphic Medial Preoptic Area

Perhaps the most widely known animal model for neural sexual dimorphism is found in the rat medial preoptic area (mPOA). The mPOA appears to be a crucial element in reproductive behavior and endocrine status of rats. In males, the mPOA plays a role in rat copulatory behavior (174), as lesions here abolish or modify normal mating behavior while testosterone propionate (TP) implants in the adult mPOA restore mating in castrates. In female rats, the mPOA has been implicated in maternal behavior, lordosis, and the cyclic release of gonadotropins. Implants of TP into the mPOA of females induces mounting and other male-like copulatory behaviors (121, 179). The mPOA receives afferents from the medial amygdala, bed nucleus of the stria terminalis, hippocampus, and septum, as well as ascending projections from serotonergic neurons. The first three of these afferents themselves display considerable sexual dimorphism, as we relate below. The mPOA is also innervated by much of the hypothalamus. In turn the mPOA projects reciprocally to all of these areas and sends descending inputs to most of the periventricular nuclei of the hypothalamus (190).

Within the mPOA of rats is a region of densely packed, darkly staining cells which is strikingly sexually dimorphic (77, 78). This region was named the sexually dimorphic nucleus of the medial preoptic area (SDN-POA). Adult male

rats have SDN-POA volumes between 2.5 and 5.0 times greater than that of females. The function of the SDN-POA in rats remains disappointingly uncertain. Lesions limited to the SDN portion of the POA have either no discernible effect on behavior (5) or only a subtle and transient effect on male mating behaviors (46). The function of the SDN-POA is much better explicated in gerbils, where there are separate medial and lateral divisions of this sexually dimorphic area (SDA). Bilateral lesions of either component eliminates mating behavior in males (218) and so do bilateral knife cuts divorcing the SDA from connections with, among other areas, the medial amygdala (219). Perhaps the best indication of the function of the SDA of gerbils is the positive correlation between volume of the "pars compacta" region and T-induced scent marking and mating behavior (206). Interestingly, this correlation seems to depend upon perinatal exposure to *both* androgenic and estrogenic steroids. As we will see next, steroid hormones can also influence the volume of the SDN-POA in rats.

Neurons of the mPOA in both male and female rats contain more estrogen receptors than any other brain region (88). There is some evidence that the immunoreactivity and concentration of nuclear estrogen receptor binding sites may be greater in females than in males (29). The concentration of androgen receptor binding sites in the mPOA may also be sexually dimorphic. While the SDN-POA within the mPOA preferentially binds estrogen in both sexes, it also accumulates testosterone and DHT, but these androgens are taken up more heavily in male rats than in females (98).

Sexual dimorphism in the volume of the mPOA has been described in many species, including rats (77, 78), gerbils (39), guinea pigs (92), ferrets (202), quail (152), macaques (10), and humans (4, 119, 199). Surprisingly, no sex differences have been found in the volume of the mouse mPOA (221) or in its density (17). The SDN-POA is significantly larger in male than female mountain voles, but there is no difference between the sexes in the closely related prairie vole—a finding which correlates with the different mating patterns of the two species (183), as mountain voles are polygynous while prairie voles are monogamous. The presence of a sexually dimorphic region within the mPOA in so many species supports the notion that these structures are evolutionarily homologous.

The Role of Steroids in the SDN-POA

The SDN-POA of rats conforms beautifully to both the organizational and the aromatization hypotheses. Adult hormone manipulations do not alter SDN-POA volume in either sex, but exposure of developing female rats to testosterone will lead to a larger, more masculine SDN-POA in adulthood, while castration of newborn males will result in a smaller, more feminine SDN-POA (77, 78). Early estrogen treatment is more effective than early androgen treatment, and early anti-estrogen treatment of males reduces the size of the SDN-POA in adulthood (56). Also, the SDN-POA in *Tfm* male rats (which inherit a nonfunctional androgen receptor yet have normal aromatase and

estrogen receptors) is equal to that of wild-type males, strong evidence that the action of estrogens alone is sufficient to masculinize the nucleus (97). So it appears that aromatized metabolites of androgen masculinize this structure. Because steroid hormones are secreted by the fetal testes during neurogenesis of the SDN-POA, and because there may be more neurogenesis in the SDN-POA of fetal males than of females (100), hormones may masculinize the SDN-POA by augmenting neurogenesis, adding a larger complement of neurons in this region in males than in females. However, as seen elsewhere in the CNS, the predominant role of hormones may be to spare neurons in the SDN-POA from programmed cell death (45).

The masculine volume of this nucleus depends on the presence of gonadal steroids between postnatal day one (P1) and P7, well after the completion of neurogenesis. Adult male volumes are significantly reduced with neonatal castration, and female volumes of the SDN-POA reach control male values with P1 treatment with TP (99). Neonatal castration also reduces cholecystokinin (CCK) immunoreactivity in the adult mPOA, the encapsulated portion of the bed nucleus of the stria terminalis, and the posterodorsal portion of the medial amygdala (189). In adult male gerbils, castration reduces the size of the mPOA overall as well as the SDA (39). There is one report of a sex difference in the efferent projections of the mPOA; the output fibers of the gerbil mPOA are more robust in males than in females (62).

The cytoarchitecture of the mPOA is also sexually dimorphic. Recall that Raisman and Field found a sex difference in synaptic subtypes of nonamygdaloid origin within the mPOA. Males possessed more axosomatic synapses and fewer dendritic spine synapses than did females, a difference that was determined by gonadal hormones during early postnatal life (161). The dendritic arbors of mPOA cells are also sexually dimorphic in Syrian hamsters: males tend to have a denser central dendritic field, while females have a more uneven and irregular distribution of synaptic fields (80).

The role of GABA activity in the masculinization of the mPOA has been investigated by several researchers. Neonatal sex differences in an enzyme important for GABA synthesis, glutamic acid decarboxylase (GAD), have been detected via immunocytochemistry in the preoptic area. The differences disappear by P15 when GAD levels drop overall (129). Sex differences in GABA were also found on P10, with males having more such immunoreactivity (65). Interestingly, treatment of developing male rats with the GABA antagonist muscimol mimics the effect of castration in the mPOA without changing endogenous steroid levels (11).

The Vomeronasal System

The sexually dimorphic vomeronasal system (VNS; Fig. 1) contains a multisynaptic circuit that begins in a sense organ, the vomeronasal organ, and ends in endocrine, motivational, and motor centers such as the hypothalamus, basal ganglia, and brain stem. Because we have at least a rough idea of the function

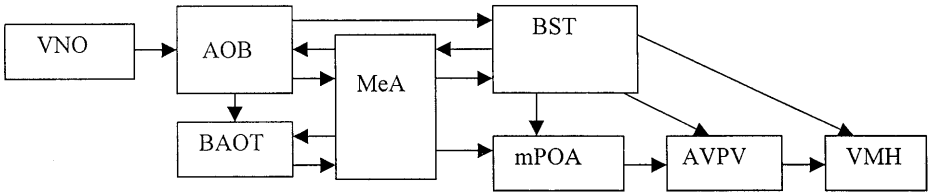

FIG. 1. A schematic diagram of the neural circuitry which receives information from the vomeronasal organ (VNO), the receptor for pheromonal signals. Sexual dimorphism has been reported for each of these neural regions in rats. The interconnected nature of this system of nuclei is obvious even though, for clarity, not all the connections between them are depicted. Abbreviations: AOB, accessory olfactory bulb; AVPV, anteroventral periventricular nucleus; BAOT, bed nucleus of the accessory olfactory tract; BST, bed nucleus of the stria terminalis; MeA, medial amygdala; mPOA, medial preoptic area; VMH, ventromedial hypothalamus.

of the VNS (integrate olfactory and pheromonal cues with other information to modulate reproductive function) and because the sexual dimorphism seems so widespread, this seems an excellent system for studies of brain sexual differentiation. The majority of VNS data comes from rodents, because they rely heavily on pheromones to regulate their reproductive behavior and to modulate their internal hormonal milieu (86). Sexual dimorphisms within the olfactory limbic system (amygdala, bed nucleus of the stria terminalis, preoptic area, and various hypothalamic nuclei) have also been found in humans and non-human primates, although the role of pheromones in human mating behavior remains controversial (e.g., 197).

The vomeronasal organ (VNO) is a tube-like pit within the nasal septum. Beneath a layer of mucus on the medial side of the VNO are the dendrites of bipolar neurons. Just as a sexually dimorphic system of brain nuclei is connected to the vocal organ in birds, an extensive system of sexually dimorphic brain nuclei is connected to the mammalian VNO. At the level of the VNO itself, there are sexually dimorphic patterns of pheromone receptor mRNA in the bipolar cell dendrites of rats (90). Furthermore, male rats have more bipolar cells than do females, but the bipolar cells of females have larger nuclei than those of males (178). The bipolar neurons project to the accessory olfactory bulb (AOB) a hemispheric, laminar structure located in the dorsocaudal quarter of the main olfactory bulb. The AOB is also sexually dimorphic, with male rats presenting greater overall volume and total number of neurons than do females (172, 180, 208). The dendritic arbors of these cells are also greater in males (31). The majority of efferents from the AOB synapse in the medial and corticomedial amygdala, and it is the sexual dimorphism of the medial amygdala which we discuss next.

The Medial Amygdala

The medial amygdala (MeA) contains a high proportion of neurons that possess steroid receptors (187) and is thought to integrate information relayed

by AOB afferents as well as inputs from the genitalia (217). Lesions of the male rat MeA disrupt the display of erections in response to distal cues from estrus females, but spare most other parameters of copulatory performance (115). The rodent MeA also plays a role in female reproductive behavior, including lordosis (156) and the modulation of endocrine events such as prolactin and luteinizing hormone surges (162). Besides its participation in reproductive behavior, the MeA has also been implicated in aggressive behavior (209) and male-typical juvenile play (132). The overall volume of the MeA is sexually dimorphic (41, 135): larger in male than in female rats. The posterodorsal portion of the MeA (MePD) contains especially high concentrations of androgen concentrating cells (184) and is 85% larger in volume in males than in females (91). Correspondingly, neuronal somata of the MePD are larger in males than in females, whereas no such sexual dimorphism is present in the postero-ventral portion of the MeA (41). Neuronal nuclei within the MeA are also sexually dimorphic in rats (196) and squirrel monkeys (30).

The functional roles of estrogen and androgen receptors in the organization of the MeA remains unresolved. While the MeA contains a substantial number of neurons that express androgen and/or estrogen receptors, the androgen receptors are found throughout the MeA, whereas estrogen receptors are localized mainly within the MePD (187). Interestingly, the overall volume as well as the size of neuronal somata in MeA are feminine in androgen-insensitive (*Tfm*) rats (41), suggesting that androgen receptors play an important role determining these sex differences. On the other hand, evidence also suggests that estrogen is involved in determining the sexual dimorphism in the volume of this structure. Nishizuka and Arai (142, 143) found that administration of EB from postnatal day 1 (P1) through P30 masculinizes MeA volume in rats. So perhaps both androgen receptors and estrogen receptors are normally required for masculine development of the MeA. The feminine state of the MeA in *Tfm* rats is unusual, as most studies of rat behavior indicate that early treatment with nonaromatizable androgens will not masculinize later behavior. However, Meaney and Stewart (132) found that implants of dihydrotestosterone (DHT) into the amygdala masculinized the juvenile play of female rats, whereas treatment with estradiol had no effect. Furthermore, they found that the juvenile play of *Tfm* rats was feminine, so it is possible that the feminine structure of the MeA in these rats is related to the feminine levels of juvenile play they display. Kerchner *et al.* (112) reported that male MeA volume is resistant to the demasculinizing *in utero* effects of stress, which reduces the size of the SDN-POA.

The extent to which the MeA is sexually dimorphic in other species is unclear. Preliminary evidence (Cooke and Breedlove, unpublished observations) suggests that there is no sexual dimorphism of MeA volume in adult male and female C57BL/6J mice. There may be functional sex differences however, since Arimatsu *et al.* (6) report that adult male mice have greater α-bungarotoxin binding capacity within the MeA than do adult females. Male values are reduced by adult castration, but they nevertheless remain greater than in control females. These results suggest that the cholinergic innervation of the

MeA is not only permanently masculinized during development, but also maintained by hormone in adulthood. Uno *et al.* measured amygdala volumes in rhesus monkeys using magnetic resonance imaging (MRI) and found that males did not differ from females in this regard (207).

Conflicting reports make it unclear whether there is also a sexual dimorphism of the amygdala in our own species. A developmental sex difference in the human amygdala was observed by Caviness *et al.* using volumetric MRI. The volume of most brain structures are uniformly scaled to the growing volume of the entire brain. An exception to this is the amygdala, which is disproportionately smaller in the female child's brain relative to the male (33). Murphy measured the volume of the MeA in 17 human brains aged 35 weeks prepartum to 94 years. Using an analysis of covariance to control for age effects and sex differences in overall brain size, Murphy (140) found no sex differences in the volume of either the right or the left medial amygdala.

Bed Nucleus of the Stria Terminalis

The next relay in the VNS is the bed nucleus of the stria terminalis (BST), which receives afferents from the MeA via the stria terminalis and directly from the AOB. The BST also receives input from the anterior amygdala (which receives inputs from main olfactory bulb). In turn, the BST projects to the POA discussed earlier, the ventromedial hypothalamus and back to the MeA (179, 222). The BST is a forebrain structure that has been variously described as part of the amygdala (52), a secondary olfactory system (101) and a subcortical olfactory system. It is also a target for gonadal steroids (40, 184) and is thought to be involved in the endocrine and behavioral control of reproduction (60), since lesions of the BST impair chemoinvestigation in male hamsters, but do not abolish copulation and intromission (159). BST lesions in ovariectomized, estrogen-primed female rats facilitate lordosis (120).

The overall volume of the BST is not sexually dimorphic, but this may be due to the complementary patterns of dimorphism within the subnuclei of this structure. Segovia and Guillamon (179) divide the BST of rodents into four main divisions according to two axes—anterior/posterior and medial/lateral. The medial posterior portion of the BST (BSTMP) is a darkly staining nucleus that has also been named the principal (32) or encapsulated nucleus (91). This region is heavily interconnected with the MePD and other limbic structures that are sexually dimorphic, express hormone receptors, or both (106). The BSTMP is sexually dimorphic, with male rats presenting significantly larger volumes (91) and greater numbers of neurons (83) than do females. Analogous sex differences have been found in the guinea pig (92), and the human brain where the nucleus is 2.47 times larger in males than in females (2).

Other regions of the BST are also sexually dimorphic, but in the opposite direction to that seen in the rest of the VNS. The lateral-anterior BST (BSTLA) has more neurons in females than in males, and neonatal orchidectomy results in a significant increase in the adult number of neurons over control males. As

expected, treatment of newborn females with androgen reduces the number of cells in the adult BSTLA to values equal to those of control males. In the medial-anterior BST (BSTMA), females have more neurons than do males, and neonatal orchidectomy increases male numbers to that of female controls. Paradoxically, treatment of females with TP causes a further increase in cell number in the BSTMA beyond that of control males and females (179). The cellular mechanisms by which these sex differences in neuronal number arise has not been determined.

Sex Differences in Neuropeptide Expression in the VNS

There are dramatic sex differences in the pattern of immunoreactivity of several neuropeptides in both the MeA and BST of rodents. Substance P-immunoreactive (ir) perikarya reside in the MePD and Substance P-ir terminals are found in the BST. Substance P staining in the MePD is more than two times greater in males than in females, and this difference depends on adult androgen concentrations: 8 weeks postsurgery, castrated males have approximately 42% smaller Substance P-ir areas within the MePD than their TP-treated counterparts. This effect of adult castration was also observed in the BST (123).

Two other peptides, CCK and vasopressin, are present within the MePD and are known to have sexually dimorphic distributions. Males have more CCK-ir cells in the MePD than do females (134). This sex difference also appears to depend on adult hormone concentrations, since castration of adult males dramatically reduced CCK-ir, and this could be reversed with testosterone replacement (189). Sex differences in arginine vasopressin (AVP) immunoreactivity within the MePD and BSTMP have been well characterized by Geert De Vries and colleagues. AVP-ir cells are sexually dimorphic within both regions, with males presenting terminal staining areas twice as large as those of females. Gonadal hormones during the early postnatal period determine the number of AVP-ir cells in the BST and MeA. AVP-ir cells contain both androgen and estrogen receptors and lose their AVP immunoreactivity after adult gonadectomy. AVP-ir staining is recovered upon adult hormone replacement (49). AVP-ir cells in the BST and MeA share the same birthdate, embryonic day 12 (E12) and E13, which is 1 to 2 days earlier than most other cells in the same area (48). But there is a sex difference in those birthdates. While there is no significant sex difference in AVP-ir cells born on E12, males have significantly fewer AVP-ir cells born on E13 than do females.

Examining human tissue, Zhou and co-workers (222) compared the volumes of vasoactive intestinal peptide (VIP) immunoreactivity within the central subdivision of the BST (BSTc) in females, male heterosexuals, homosexuals, and male-to-female transsexuals. They found that the volume of the BSTc was sexually dimorphic and was feminine (i.e., smaller than in normal men) in male-to-female transsexuals. This difference appears to be independent of sexual orientation, as BSTc volumes in male homosexuals were masculine and

the sexual orientations within the transsexual sample included homosexual and heterosexual subjects. Further, the degree of VIP immunoreactivity may be independent of adult hormone environment, as a few males orchidectomized for medical reasons showed BSTc volumes in the upper range for nontranssexuals and homosexuals. The BSTc appears to be located rostral to the BST enc as described by Allen and Gorski (2) and therefore may represent a second region within the BST that is sexually dimorphic in humans.

Together, the MeA and BST are described by Alheid *et al.* (1) as components of the "extended amygdala." This description is based on the somatotopic symmetry between subnuclei in each of the two areas (i.e., medial maps to medial, lateral to lateral). The MeA and BST are connected by the stria terminalis which arches dorsorostrally with the stria medullaris to the basal forebrain. The implication of this anatomic pairing is that parallel processing may occur within the extended amygdala, as each MeA subnucleus maps onto its own BST nucleus, and back again (1). The pervasive sexual dimorphism in both regions seems to confirm this notion that they are closely related neural systems.

Anteroventral Periventricular Nucleus (AVPV)

The AVPV is a small cluster of cells rostral to the mPOA. It may mediate the feedback of ovarian hormones to control the release of GnRH by the hypothalamus. The majority of AVPV efferents project through the periventricular zone of the hypothalamus and form terminal fields in the periventricular nucleus, the arcuate nucleus, and the parvicellular regions of the paraventricular nucleus. The AVPV also projects strongly to the medial zone of the mPOA (82). The AVPV is sexually dimorphic, with females presenting greater volumes and cell densities than males (17). These differences are dependent on neonatal sex steroids: orchidectomy of male rats and TP treatment of females abolishes the sex difference. Neonatal androgen treatment of females reduces the number of cells within this nucleus (188).

Correlated with the sex difference in the number of Nissl-stained cells in the AVPV are sex differences in the number of cells expressing various neuropeptides. Females also possess greater numbers of dopaminergic neurons in AVPV (188), as measured by the number of tyrosine hydroxylase (TH)-ir neurons. TH is a rate-limiting enzyme for catecholamine metabolism and therefore an indirect measure of dopaminergic terminals. Simerly and colleagues found that TH immunoreactivity was feminized in male estrogen receptor knockout mice but normal in affected *Tfm* males. These results suggest that dopaminergic neurons are masculinized by estrogen receptors alone, independently of androgen receptors (191). For other neuropeptides, the sex difference in this region seems reversed from those above. Male rats have more AVP-ir cells and enkephalin-ir cells (192) in the AVPV than do females.

The volume of the AVPV in mountain and prairie voles is sexually dimorphic and, as in the mPOA, the magnitude of the difference seems to reflect aspects of their behavioral ecology. Mountain voles are a polygamous species, while the

closely related prairie voles are monogamous. The magnitude of the sex difference is much greater in the mountain vole, as the AVPV is usually undetectable in males. Sex differences in the prairie vole AVPV are comparable to those of the rat (183).

Ventromedial Hypothalamus

The ventromedial hypothalamus (VMH) is a sexually dimorphic nucleus that receives input from the VNS, including the MeA, lateral septum, BSTMP, mPOA, and AH. In turn, the VMH projects to the suprachiasmatic nucleus, the supraoptic nucleus, the median eminence, the arcuate nucleus, as well as the midbrain central grey and other brain stem nuclei. The VMH appears to be involved in the control of feminine reproductive behavior, including maternal behavior and lordosis in rats (121).

The first reports of sex differences within the VMH came from Dörner and Staudt (57), who found that the cellular nuclei of VMH neurons were larger in female than in male rats. This difference could be reversed with P1 castration, which also increases the frequency of lordosis in males given estrogen and progesterone in adulthood. The overall volume of the VMH, however, is larger in male rats than in females. Castration of males on P1 but not on P7 reduces the volume of the VMH to that of control females (57).

As with MeA, the ventrolateral VMH in males has significantly more shaft and spine synapses than that of females (126). No sex differences were found in the dorsomedial region of the VMH. Neonatal castration and hormone treatments reverse the sex difference in synaptic pattern, as was seen in the MeA. Investigators have also found sex differences in neuropeptide expression within the VMH. Greater CCK immunoreactivity is seen in adult male rats (134). Also, CCK levels fluctuate with the estrous cycle, with lower levels of CCK found at the time of estrous.

The Role of Steroids in Sexual Differentiation of the VNS

We have touched upon steroid effects on each of the components of the VNS, but it is worthwhile to step back and review them at this point (Table 1). Most of the sex differences in the VNS have been experimentally reversed by early postnatal orchidectomy of males and/or steroid treatment of females.

For example, estradiol administered to female rats on P1 masculinizes the number of AOB mitral cells, whereas the nonaromatizable metabolite of testosterone, dihydrotestosterone (DHT), does not (154). AOB neurons project not only to the MeA, but to several other nuclei within the limbic system. The most rostral target is the bed nucleus of the accessory olfactory tract (BAOT). This cluster of large, darkly staining cells lies rostroventral to the MeA, near the surface of the brain (1). BAOT neurons also receive inputs from the MeA (114) and they project to the AOB and to the preoptic area (POA) (52). The function of

TABLE 1

Sexual Dimorphisms in the Rat Brain Vomeronasal System and Effects of Early and Adult Hormone Manipulations

		Perinatal manipulation		Adult manipulation		
VNS nucleus	Controls	Male castration	Female androgen	Male castration	Female androgen	References
VNO						
volume	M > F	↓	↑			(178)
bipolar neurons						
cell number	M > F	↓	↑			(178)
nuclear size	F > M	↑	↓			
AOB						
volume	M > F	↓	↑			(180)
mitral cell size	M > F	↓	↑			(208)
MeA						
overall volume	M > F[a,b]	↓[a]	↑[a]	↓[b]	↑[b]	[a](135),[b](42)
MePD						
volume	M > F					(42)
soma size	M > F			↓	↑	(42)
MePV						(42)
volume	M = F			No effect	No effect	
BST volume	M = F	No effect	↑			(179)
MP volume	M > F	↓	↓	No effect	No effect	(179)
MA volume	M < F	↑	↓			
LA cell number	M < F	↑	↓			(83)
SDN-POA						
volume	M > F[a]	↓[a]	↑[a]	No effect[b]	No effect[b]	[a](77),[b](167)
cell density	M > F	↓	↑			(78)
AVPV volume	M < F	↑	↓			(43)
cell density	M < F	↑	↓			(198)
VMH volume	M > F	↓	↑			(127)

Note. Where the results of two different studies are reported on a single line, superscripts denote which reference (right) reported each effect.

the BAOT is unknown, but its proximity to the MeA and heavy projections to the POA suggest that it is involved in the processing of inputs from the AOB and modulation of integrating centers such as the POA. The BAOT is sexually dimorphic—male rats have more neurons and glial cells than do females. This difference can be reversed with neonatal orchidectomy and androgen treatment (37).

Ultrastructural patterns within the MeA also differ between male and female rats. Nishizuka and Arai (144) found that males possess more shaft synapses in the medial part of the molecular layer (i.e., the halo of dendrites around the central core of cells) and more spine synapses in the ventral part of the molecular layer. These differences are reversible with early orchidectomy of males and early androgen treatment of females. The same authors grafted female neonatal MeA tissue into the eye of an orchidectomized female and treated the female with estrogens. In contrast to the vehicle-treated control group, the EB-treated grafts possessed significantly more shaft synapses (143).

TABLE 2

Soma Size of Neurons in the Posterodorsal Portion of the Medial Amygdala (MePD) in Male and Female Rats That Were Subjected to Either Sham Surgery or Gonadectomy at 60 Days of Age and Sacrificed 30 Days Later

	Adult treatment		
	Sham + blank capsule	Gonadectomy + blank	Gonadectomy + testosterone
Male	161.0 ± 5.9 (6)	121.3 ± 10.9 (5)	166.2 ± 4.1 (5)
Female	133.7 ± 10.5 (7)	116.0 ± 7.5 (5)	161.2 ± 7.5 (7)

Note. Means (μm^2) and standard errors of the means are presented. The number of animals per group is indicated in parentheses. Among sham-operated animals, MePD neurons were significantly larger in males than in females ($p = 0.05$, two-tailed, t test). Castration and implantation of a blank (empty) Silastic capsule significantly reduced the size of these neurons in males only, eliminating the sex difference. A month of testosterone treatment through 40-mm Silastic implants significantly increased the size of MePD neurons only in females, again eliminating the sexual dimorphism. A two-way analysis of variance among the four gonadectomized groups revealed a significant main effect of testosterone treatment ($F_{(1,18)} = 21.5$; $p < 0.0003$), but none for sex ($p > 0.50$).

Orchidectomy and hormone treatments on P1 cause familiar sex difference reversals within the rat BSTMP. Aromatizable androgens can increase the volume of the nucleus beyond that of control males, and neonatal orchidectomy demasculinizes the BSTMP in males (50). This pattern is in part due to differential rates of cell death. Chung *et al.* found more pycnotic cells in the BSTMP in females on P5 and P6 than in males (34).

However, recent evidence in our laboratory (42) suggests that adult gonadal steroids may in some cases determine the sexually dimorphic characteristics of the VNS. For example, the size of neurons within the MePD is sexually dimorphic in adulthood, with male rats having larger neurons than females. Castrating adult male rats causes the MePD neurons to shrink significantly, making the measures comparable to those seen in females (Table 2).

Similarly, Gomez and Newman (76) used Golgi-stained material to show that the MeA neurons of intact, castrate, and DHT-treated adult male Syrian hamsters also undergo structural changes in the number of dendritic branches and segments, as well as somatic area, after androgen manipulations in adulthood. These results emphasize the important but often overlooked possibility that gonadal hormones in adulthood may determine the sexually dimorphic parameters of a neural system. In other words, despite the implicit message of the original organizational hypothesis, it is sometimes possible for steroid hormones to "activate" major structural reorganization of the adult brain.

Hippocampal Formation Sex Differences

The hippocampus has long been thought to mediate aspects of spatial learning, and in several species males are somewhat better at such tasks than

are females (171, 186) so it is perhaps not surprising to learn that there is sexual dimorphism in the hippocampal formation.

Wimer and Wimer (215) examined sex differences in the granule cell layer of the hippocampus in six different strains of mice. They found differences between strains in the number of granule cells. Among strains that have a higher number of granule cells, females have significantly fewer cells than do males. The three strains that have fewer granule cells were sexually monomorphic for this measure. Cell density varied from strain to strain with no relation to the total number of cells. In every strain, cell density was greater in males compared to females of the same strain.

Roof and Havens (171) tested the performance of adult male and female Sprague–Dawley rats in a Morris water maze task. Males learned the task more rapidly, while perinatally androgenized females performed better than either control males or females. This difference in behavior may have a morphological correlate in the dentate gyrus. The dentate gyrus granule cell layer (DG-GCL) width was larger in control males than in control females and was laterally asymmetrical (larger on the right) in males only. The width of the DG-GCL in perinatally androgenized females fell between that of control females and males and was laterally asymmetric, as in males. Maze performance was positively correlated with the width of the right DG-GCL. Roof (170) reported in a subsequent study that these sex differences in DG-GCL were also present in prepubertal rats, indicating that the gonadal hormone surge during puberty is not responsible for this sexual dimorphism. The hippocampal formation also appears to be lateralized in humans. The right hippocampal formation is larger than the left in adult human males which served as controls in a study of schizophrenics (72), and in both sexes in a study of developing humans (74), so hippocampal asymmetry may be an overlooked yet widespread phenomenon. Functional MRI studies indicate that the right hippocampus, not the left, is activated when people are mentally navigating a "virtual" city (122), which supports both the notion that the hippocampus plays a role in spatial navigation and that sexual dimorphism of the right hippocampus in rats may be related to sex differences in their spatial learning ability.

As befits a brain region implicated in learning, the hippocampus appears to be sensitive to experience. Juraska (107) found that female rats raised in a complex environment had greater dendritic intersections per neuron in the dentate than did females raised in isolation. Male rats failed to show the same response. If anything, the direction of response seemed to be reversed in males. Morse, Scheff, and DeKosky (139) looked at the effect of either testosterone or estrogen treatment in gonadectomized or sham operated male and female Sprague–Dawley rats on the reactive outgrowth of the commissural afferent fibers in the dentate gyrus after removal of the entorhinal cortex. Male and female controls had identical sprouting responses. Only females were affected by gonadectomy, resulting in less fiber outgrowth. Hormone replacement therapy (testosterone or estrogen) brought outgrowth back to control levels in females, whereas fiber outgrowth in males was unaffected by testosterone or estrogen.

Gould, Westlind-Danielsson, Frankfurt, and McEwen (79) examined the

effects of thyroid hormone on sex differences of the CA1 and CA3 regions of the rat hippocampus, as well as projecting granule cells of the dentate gyrus. Only two sex differences were found. In the pyramidal cells of CA3 the density of apical dendritic spines was greater per unit area in males and the number of primary dendrites was greater in females. The latter finding seems to be the result of more basal dendrites, since no sex difference was found in apical dendrites. These two sex differences were unaffected by neonatal thyroid hormone treatment.

Sherry, Galef, and Clark (185) measured hippocampal volume of male and female Mongolian gerbils. They hypothesized that intrauterine position (i.e., whether an animal had two males, two females, or an animal of each sex as neighbors *in utero*) might influence the size of the hippocampus. The volume of the hippocampus was larger in males that were randomly selected with regard to uterine neighbors compared to randomly selected females. But intrauterine position also had a significant effect, so that if one sampled only females that had resided between two males *in utero*, then the sex difference was lost.

Jacobs and Spencer (96) examined the hippocampus of two species of kangaroo rats: Merriam's kangaroo rats and bannertail kangaroo rats. Merriam's kangaroo rats store seeds in scattered locations, requiring considerable spatial memory skills, whereas bannertail kangaroo rats return food to one central cache. As expected, Merriam's kangaroo rats have a larger hippocampus. In both species, males are polygamous and have a larger hippocampus than do females. Jacobs and colleagues (95) compared the size of the hippocampus of two different species of vole. In the polygamous meadow vole the hippocampus was larger in males than in females, while in the monogamous pine vole, no sex differences were observed. This result suggests that the hippocampus aids the spatial memory of male voles that must remember the location of multiple sexual partners. Reboreda, Clayton, and Kacelnik (166) examined the hippocampi of different species of cowbirds which follow different nesting strategies. Some species are nest parasites (laying eggs in nests of other species, for the foster parents to raise), while others are not. The shiny cowbirds parasitize more than 200 different hosts, again requiring great spatial memory. The hippocampal volume was greater in these birds than in the bay-winged cowbird, which is not a nest parasite. Only the female inspects nests in the shiny cowbirds in contrast to the screaming cowbirds in which both sexes inspect nests. Although the screaming cowbirds had a larger hippocampus than the bay-winged cowbirds, the females did not have a significantly larger hippocampus than the males, but such a sex difference is observed in the shiny cowbirds, in which only females scout for nests. After observing no sex difference in spatial learning capacity in black-capped chickadees, hippocampus size was compared between the sexes. Behavior seems to be predictive of hippocampus size since no sexual difference was found (155).

Physiological sexual dimorphism of the hippocampus has also been reported. CA1 hippocampal evoked field potentials is affected by adult gonadal steroid administration (201). In males, 17-β-estradiol enhances the evoked field potentials, and in females, testosterone has the same effect (70). To assess sex

differences in hippocampal synaptic plasticity, Maren, De Oca, and Fanselow (125) examined perforant path-dentate granule cell long-term potentiation (LTP) as measured by excitatory postsynaptic potentials slope. LTP varied with sex: males exhibit more LTP than do females in 35-day- and 60-day-old groups. Data indicate a strong correlation with LTP and levels of *N*-methyl-D-aspartate receptor activation. Since contextual fear conditioning may be mediated by LTP, the same team of researchers examined conditional freezing in both sexes following Pavlovian fear conditioning (context served as a cue for footshock). As expected, males exhibited more conditional freezing than did females. A third experiment showed that the enhanced fear conditioning in males was specific to contextual conditional stimuli—specific tone-shock conditioning showed no sex difference in freezing and consisted of a more rapid rate of conditioning. Bronzino *et al.* (28) demonstrated that 30-day-old male and female Sprague–Dawley rats exhibit different enhancement profiles in magnitude and duration of LTP. Neonatal isolation alters LTP profiles differentially from one sex to the other: isolation enhances the posttetanization response only in males.

Other Sexually Dimorphic Neural Systems

A striking extension of work on brain sexual dimorphism is found in certain whiptail lizard species which consist exclusively of females. These animals reproduce by parthenogenesis, but closely related species reproduce sexually. In the sexually reproducing species you can find sexual dimorphism in the anterior POA which is reminiscent of the sex difference in the rat SDN-POA (i.e., is larger in males than in females), and a converse case in that the ventromedial hypothalamus (VMH) is larger in females (211). The extent of the dimorphism varies depending upon the season of the year animals are examined, which suggests that adult hormone levels may contribute to the sex differences in neural structure and behavior. Interestingly, in the closely related unisexual (all female) species, *Cnemidophorus uniparens,* the volume of the POA and VMH nuclei are in the range of females from the sexually reproducing species and do not seem to vary seasonally.

Weakly electric fish such as *Sternopygus* produce electric fields with highly modified muscle cells (electrocytes) and then use specialized skin receptors (electroreceptors) to detect perturbations in that electric field. A medullary pacemaker nucleus derives spinal electromotoneurons which innervate the electrocytes. The production and detection of the fairly constant, sinusoidal electric organ discharge (EOD) provides the fish with information about objects in the turbid water they normally inhabit. But the EOD also serves as a communication mechanism between individuals, because the frequency of the EOD in males decreases when they reach mating condition. Harold Zakon and colleagues have studied the role of androgens and estrogens in inculcating these sex differences in behavior. Treatment with the nonaromatizable androgen dihydrotestosterone (DHT) lowers the EOD frequency by prolonging the duration of the action potential produced by the electrolyte. Apparently the

androgen treatment alters the action potential by affecting the kinetic properties of the sodium channels (slowing the inactivation time) mediating the action potential (61). In this same species, estrogen can increase the EOD frequency (58), augmenting the sex difference. Since both androgen receptors and estrogen receptors are found in the electrolytes, it's possible that steroids are acting directly on these cells to alter their expression of ion channels and therefore their electrical properties. However, androgens increase and estrogens decrease the firing pattern of the medullary pacemaker neurons and the spinal electromotoneurons which drive the EOD in another electric fish, *Apteronotus leptorhynchus*. The steroids have this effect even if the connections between the medullary cells and the spinal cord are severed (177). Thus it may be that steroids are acting at several different levels to affect these systems, a common theme in this field.

In the midshipman fish (*Porichthys notatus*), studied by Andrew Bass and colleagues, the production of male-typical mate calling features another twist. There are two different types of adult males in this species. One morph, known as Type I males, are larger than females and produce a sonic signal (a "hum") to attract females to the nest site for spawning. These males avidly protect their nesting territories. Another male morph is smaller than Type I males and neither defends a territory nor vocalizes, but still manages to sometimes fertilize eggs deposited by a female at a Type I male's site. These latter males are known, appropriately enough, as "sneaker" (or, less judgmentally, Type II) males. In a system that is reminiscent of the EOD system of electric fish, the sonic signals of the midshipman fish are controlled by motoneurons which receive phasic excitation from pacemaker neurons in the lower brainstem and upper spinal cord (12). The size of the individual neurons in this circuit increases as the animals grow from juvenile to adult status, but the trajectories are quite different in Type I and Type II males. The morphology of POA neurons which are immunoreactive for GnRH are also different in Type I and Type II males (81), so the different morphs could be the result of differences in the amount or pattern of gonadal steroid responses to gonadotropins.

SOME GENERAL PRINCIPLES OF SEXUAL DIMORPHISM IN THE NERVOUS SYSTEM

Perhaps the single most obvious conclusion about sexual differentiation of these neural systems in animals is the pivotal role of steroid hormones. In every case discovered so far, one can manipulate the sexual dimorphism in the nervous system by manipulating steroid hormones. The fascinating caveat is that, in birdsong systems, it has so far been possible to masculinize females with early estrogen and adult androgen treatment but it has been singularly difficult to block masculinization of males with any steroid manipulation (8). If the failure to prevent masculinization in males turns out to be due to inadequacies in methodology, then the role of steroid hormones in CNS sexual differentiation seems secure for mammalian and avian species. Conversely, if some

nonsteroidal mechanism is responsible for masculinization of the male songbird brain, then it will be fascinating to ask why females nevertheless respond to steroids. Furthermore, it will be important to ask whether there are other, nonsteroidally mediated sexual dimorphisms in other vertebrates. Finally, if bird brain sexual differentiation is induced by a fundamentally different mechanism than that used in mammals, questions will arise about the evolutionary history of this divergence.

Another lesson to be gained from these models is the versatility of steroids in terms of cellular mechanisms. Steroid hormones have been found to alter sexual dimorphism by affecting neurogenesis (75, 100), cell death or apoptosis (44, 116, 146), cell migration (20, 182), neuronal somatic growth (24, 42), dendritic growth (53, 66, 117), synapse formation (118, 128), synapse elimination (103, 104), and neuropeptide expression (47, 134). This multiplicity of cellular mechanisms affected by steroids suggests two ideas which are not necessarily contradictory. First, that steroids have been affecting the nervous system for a considerable stretch of evolutionary history. Second, because these cellular mechanisms (mitosis, migration, apoptosis, somatic growth, etc.) almost certainly evolved prior to steroidal signaling systems, it appears that natural selection can readily intercalate a steroidal-mediation step into almost any cellular process. Perhaps the wide variety of cellular mechanisms affected by steroids, and the many systems affected by steroids is simply a result of the very powerful, gene regulatory capacity of these hormones.

Another common principle suggested by these systems is that steroid hormones rarely, perhaps never, affect a neural system by altering only a single parameter in a single locus. Rather, as the birdsong, frogsong, SNB, and VNS systems indicate, it is common for androgens to induce changes in many different levels of the nervous system. Furthermore, steroid receptor expression is also found not just in one nucleus, but nearly all the nuclei in these sexually dimorphic networks. From this perspective, the SDN-POA, which was the first macroscopic sexual dimorphism found in the rat brain, and which was therefore studied for some time in isolation, might more properly be seen as part of a network of sexually dimorphic nuclei connected to the vomeronasal system. If so, then perhaps the failure to detect a robust behavioral effect of SDN-POA lesions may be due to failure to test for vomeronasal organ-related behaviors. The effects of SDN-POA lesions in gerbils is certainly consistent with this idea.

Some of the most interesting amendments to the original vision of the organizational hypothesis are cases where adult hormone treatment alters the structure of the nervous system. To date these changes have all been temporary, fading when the steroid treatment is withdrawn, but they are in concert with other neuroscience reports that portray the adult brain as retaining at least a portion of the plasticity seen in development. The most prominent example is the canary birdsong system, where androgen treatment of adult females partially masculinizes the brain structure and causes the female to sing. Adult androgen treatment can also alter the size of neurons in the SNB and the MePD. It seems reasonable to think that more examples of adult

steroid treatment altering neural structure are waiting to be described. Perhaps the most important single message concerning the effects of adult hormone manipulations is that some neural sexual dimorphisms require steroid hormones *both* during development and in adulthood to display sex differences to the greatest extent. This conclusion has implications for study of sexual dimorphism in the human brain, the topic we address next. A sexual dimorphism in the adult human brain could easily be a result of circulating gonadal steroids alone and might have nothing to do with early, fetal steroid action. Furthermore, the decline in androgen secretion in aged men, the use of steroids for birth control in women, and the issue of whether to implement estrogen therapy in postmenopausal women, may all have ramifications for brain structure and function.

SEXUAL DIFFERENTIATION OF THE HUMAN BRAIN

The question of whether steroid hormones also masculinize the developing nervous system of humans cannot yet be given a unequivocal answer (38). There are sexual dimorphisms in the human brain, to be sure, but we do not yet know whether steroid hormones play any role in their development. Because the nervous system is plastic, any sexual dimorphism seen in the adult brain could be the result of differences in experience, either during development or in adulthood, rather than as a direct result of fetal steroid action. Obviously a sexual dimorphism present at birth could not be due to sex differences in experience or social stimulation, but there are few or no such examples at present. One dimorphism present at birth is the sex difference in the weight of the human brain (150, 151) and because it is mirrored by the sex difference in body weight, it may be the result of steroid hormone action, but it seems unlikely to be a *direct* action of steroids. More likely, testicular androgens masculinize the secretion of other factors such as growth hormone or its companion factors to give males a larger body and brain. Because the effect does not seem to be specific to the nervous system, it seems unlikely that the sex difference in overall brain weight can account for the sex differences in human behavior. Otherwise, the only indication of sexual dimorphism in a human brain region before birth is a report that the fetal corpus callosum has a different shape in males and females (51). But because the very existence of a sexual dimorphism in the adult human corpus callosum remains in dispute (e.g., 15) it would be unwise to conclude that the structure is sexually dimorphic in prenatal humans based on a single report.

The sexually dimorphic brain structure that has been best studied in humans is the SDN-POA as described by Dik Swaab and colleagues (199). They find that males have a larger SDN-POA, with more neurons, than do females, but this sex difference in neuronal number is not detectable in children younger than 6–10 years of age (94). Allen and Gorski (4) examined the human hypothalamus and concluded that the area called SDN-POA by Swaab and Fliers would be more appropriately named the interstitial nucleus of the anterior hypothala-

mus (INAH) numbered 1 to distinguish it from three other INAHs. Allen and Gorski reported that INAH-2 and INAH-3 were sexually dimorphic in adult humans, but they could not replicate the sex difference in INAH-1 (=SDN-POA of Swaab and Fliers). Apparently replication is always going to be difficult in human brain measures, because LeVay (119) was unable to replicate the sex difference in the size of either INAH-1 or INAH-2. He did see a sex difference in the size of INAH-3, replicating Allen and Gorski's report, and further reported that INAH-3 was smaller in homosexual men than in heterosexual men, an "orientation dimorphism" which we will discuss later. But no one has examined the size of INAH-2 or -3 in human development, so we do not know whether the sexual dimorphism is present at birth (and therefore likely to be engendered by fetal steroids) or arises later in life (and therefore could be due to either social or steroidal influences). The conflicting reports concerning sexual dimorphism in the human brain indicate one important point—either sexual dimorphism is more subtle in the brains of humans than of other animals and/or the robust sexual dimorphism found in non-human brains is due in part to the homogeneity of subjects possible with laboratory species.

Another strategy for asking whether fetal steroids affect the human brain is to study sex differences in human behavior and ask whether inadvertent exposure to fetal hormones alter sexually dimorphic behaviors. Unfortunately, the results of such studies offer much argument and little resolution. Females are occasionally exposed to androgen *in utero,* typically due to congenital adrenal hyperplasia (CAH) which causes the adrenal glands to overproduce androgens. Such girls do behave more like boys, showing more rough and tumble play and tom-boy behaviors than other girls (13). As women, CAH patients usually are sexually attracted to men, but are more likely to be attracted to women than are non-CAH women (59). However, because the CAH condition also masculinizes the appearance of the genitalia at birth, the masculine behaviors of CAH females could theoretically be due to differences in their social experience because of confusion about their gender on the part of their parents, family, and/or peers.

Similarly, completely androgen insensitive XY individuals, who look like normal females externally, display feminine spatial learning behavior and verbal behavior, and are sexually attracted to men (137), but that could be due to either the insensitivity of their brains to androgen or their unambiguous upbringing as girls. Androgen insensitive humans do offer us one firm conclusion about the role of steroids in masculinizing the developing human brain. If, as in rodents, the aromatized metabolites of androgen masculinized the developing human brain, then we would expect androgen insensitive humans to display masculine behaviors despite their feminine exterior. Androgen insensitive rats present such a mismatch—feminine exterior phenotype, but a masculine SDN-POA (97), and a refusal to display feminine lordosis behavior (149). The masculine nervous system and behavior of androgen insensitive rats underlines the importance of estrogen receptors for sexual differentiation of behavior in this species. Conversely, the feminine behavior of androgen insensitive humans indicates that aromatized metabolites of androgen cannot be

playing a major role in masculinizing the human brain, either because steroids have no effect on the developing human brain or because steroids act through androgen receptors themselves to exert such an effect. It is also possible that in humans, androgen receptors must be functional for estrogen receptor activation to be effective or to occur at all. For example, what if androgen receptors must be activated to increase aromatase activity, ensuring that subsequent androgen is converted to estrogen? Then androgen-insensitive humans might lack the aromatase to stimulate estrogen receptors. In rats, at least, estrogen receptors alone seem to masculinize the brain. Despite the feminine behavior of androgen-insensitive XY humans, there is a study that indicates that fetal estrogens can masculinize the human brain. Melissa Hines found that women who had been exposed to the estrogen diethylstilbestrol (DES) *in utero* showed greater evidence of cognitive lateralization (for a dichotic listening task and a visual search task) than their non-DES-treated sisters (93). As males are also more lateralized than females in such tasks, the DES seems to have slightly masculinized these women. But the effect is small and the measure cannot be readily related to most human sex differences in behavior, so estrogen may be making only a small contribution to human neural sex differences.

Thus there has yet to be any conclusive demonstration that prenatal steroids can masculinize the human brain. On the other hand, a case that was said to prove the *unimportance* of fetal androgens for masculinizing behavior seems to have collapsed. An 8-month-old male child lost his penis in a surgical accident and, starting a year or so later, was surgically castrated, then dressed and reared as a girl. Early reports indicated the subject had, at 7 years of age, become a well-adjusted girl while her identical twin brother seemed a well-adjusted boy (136). Since the two had presumably equivalent fetal androgen exposure, the outcome was seen as emphasizing the importance of social upbringing over fetal steroids for sexual differentiation of human behavior. However, a recent follow-up study of the patient revealed a very different picture (55). By age 14, the child refused further feminizing surgery or estrogens and began living as a boy. Now in his 30s, the subject is happily married to a woman and father of three step-children. He insists that he always felt more comfortable with boyish activities and interests, despite what his parents reported to the original researchers (36). There is no way to determine whether this man's sexual orientation and other behaviors were made masculine by prenatal androgen, but there seems little doubt that the subject was not made feminine by social influence.

We mentioned earlier a report of an "orientation dimorphism" in the human brain—the smaller INAH-3 in homosexual men compared to heterosexual men (119). Homosexual men have also been reported to have a larger suprachiasmatic nucleus than heterosexual men (200) and to have a larger anterior commissure than heterosexual men (3). It has been pointed out that these measures, because they are made in adulthood, cannot tell us whether the brain dimorphism preceded, and therefore might possibly have caused, the differences in sexual orientation (22). It is possible with noninvasive brain imaging technology to measure the size of the anterior commissure in individu-

als before and after the development of overt sexual orientation, but such a project is so impractical that it will probably never take place. The more vexing problem is that none of these orientation dimorphisms have been replicated. Neither have there been any reports disputing the claims. To the best of our knowledge, no one has yet assembled the tissue to even attempt to replicate LeVay's findings, which speaks to the extreme difficulty of conducting such research, despite its high intrinsic interest for the public.

There have been reports of several physiological differences between homosexual and heterosexual men that could reflect nervous system involvement. For example, homosexual men are, on average, shorter than heterosexual men (18), will have undergone puberty at an earlier age than heterosexual men (124) and will have more symmetrical left-versus-right fingerprint patterns than heterosexual men (85). Homosexual men are also more likely to have older brothers than are heterosexual men, and since the most important variable is how many sons their mother carried before them (rather than how many older brothers grew up in their household), these data suggest a maternal effect on the developing fetus (16). For lesbians, the only report of a physiological difference so far is the finding that otoacoustic emissions (faint clicks emitted from the tympanic membrane either spontaneously or in response to click presentation) are more masculine (i.e., quieter) in lesbians compared to heterosexual women (130). Because a similar, freemartin-like effect is seen in the female twins of boys, this difference in otoacoustic emissions in lesbians could conceivably be due to fetal androgen exposure. While these physiological differences are consistent with the hypothesis that homosexuals and heterosexuals were exposed to different levels of fetal androgen, they do not by any means prove the hypothesis.

Despite the fact that there is not yet any conclusive proof that fetal steroids directly masculinize the human brain, the rampant masculinizing effect of androgen during early brain development of other vertebrates makes it seem likely that at least some such influences remain in our species. The challenge remains how to prove or disprove such a mechanism persists. But even if it turns out that fetal steroids have little or no direct effect on the human brain, there is no doubt that adult steroid manipulations alter human behavior and both the behavior and the neural structure of other species. So it will certainly be important for future researchers to explicate how and where steroid hormones alter the adult human brain. At the very least, this question should be answered to help us deal with issues of steroid replacement therapy in postmenopausal women and aging men.

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