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Sexual Differentiation of the Brain: A Model for Drug-Induced Alterations of the Reproductive System

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The process of the sexual differentiation of the brain represents a valuable model system for the study of the chemical modification of the mammalian brain. Although there are numerous functional and structural sex differences in the adult brain, these are imposed on an essentially feminine or bipotential brain by testicular hormones during a critical phase of perinatal development in the rat. It is suggested that a relatively marked structural sex difference in the rat brain, the sexually dimorphic nucleus of the preoptic area (SDN-POA), is a morphological signature of the permanent or organizational action of estradiol derived from the aromatization of testicular testosterone. The SDN-POA of the male rat is severalfold larger in volume and is composed of more neurons than that of the female. The observation that the mitotic formation of the neurons of the SDN-POA is specifically prolonged has enabled us to identify the time course and pathway of neuronal migration into the nucleus. Study of the development of the SDN-POA suggests that estradiol in the male increases the number of neurons which survive a phase of neuronal death by exerting a neurite growth promoting action and/or a direct neuronotrophic action. It may not be possible to extrapolate this trophic effect of estradiol to all other structural sex differences since in the anteroventral periventricular nucleus, steroid exposure reduces the number of immunohistochemically defined dopaminergic neurons. Finally, although it is clear that gonadal hormones have dramatic permanent effects on the brain during perinatal development, even after puberty and in adulthood gonadal steroids can alter neuronal structure and, perhaps as a corollary to this, have permanent effects on reproductive function. For example, in the lightly androgenized rat which exhibits the delayed anovulation syndrome, exposure to estrogen prepubertally delays the onset of ovulatory failure, whereas estrogen exposure peri- or post-pubertally has an inhibitory effect on ovulation. Although the brain may be most sensitive to gonadal hormones or exogenous chemical factors during perinatal development, such sensitivity does not appear limited to this period.

Introduction

The developing central nervous system is particularly sensitive to hormones, drugs, and environmental chemicals, and the specific process of the sexual differentiation of the brain is a valuable model system for the study of the chemical modification of its development. As an important physiological process, much is known about sexual differentiation of the brain, and mechanistic studies are now becoming possible experimentally. Moreover, exposure to drugs and environmental chemicals, certainly if they have hormonal or anti-hormonal activity, may well modify this fundamental process with permanent changes in reproductive physiology or behavior the consequence.

It is appropriate and necessary to include a consideration of the sexual differentiation of the brain in a symposium on reproductive toxicology, since it is clear that the brain is an essential component of the reproductive system. The brain is a target tissue for the action of gonadal hormones both during development (*1–4*) and during adult life (*5,6*). These hormones clearly regulate reproductive behavior (*5,7*), and play a critically important role in the regulation of the release of the various reproductively active hypothalamic, and thus, pituitary hormones (*5*). In the female rat, for example, ovulation is a neural process. The plasma titers of ovarian estradiol attained on the afternoon of vaginal proestrus, exert what is generally called a facilitatory or positive feedback action on a neural system which itself has a potential 24-hr periodicity (*6,8,9*). The result of the interaction between estradiol and this cyclic neural system is an increase in the release of luteinizing hormone-releasing hormone (LHRH) (*10,11*) which then acts on the pituitary gland now sensitized to this hypothalamic hormone also by exposure to estradiol (*12,13*). The net result is the proestrous surge of luteinizing hormone (LH) which induces ovulation. In other species such as the rabbit and cat, the reproductive function of the brain is even more explicit. These animals are reflex ovulators (*14*) and release LH in re-

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sponse to an increase in the release of LHRH triggered by the stimuli of coitus. Although it has been argued that in the rhesus monkey, the brain plays only a permissive role in the process of ovulation by releasing regular pulses of LHRH (*15*), there is ample evidence in the primate that the brain does indeed regulate ovulation and more actively (*16*).

Although it is generally accepted that gonadal steroids exert their effects on nerve cells through the classical receptor mediated genomic mechanisms, for the purposes of this discussion it is helpful to consider two very general mechanisms of steroid action. In the adult, steroids produce a temporary modification in neuronal function. Although that change may be inhibitory or excitatory, this transient action of steroids has been labeled activational (*17*). In contrast, during certain periods of development and perhaps even in adulthood as will be discussed below, steroids have the ability to change permanently neuronal function, this is called their organizational action (*17*). It is this organizational action of steroids which is the focus of the present discussion.

Sexual Differentiation of the Brain

The general tenet of the concept of the sexual differentiation of the brain is that the functional and more recently recognized structural sex differences which do exist in the brain are not due directly to sex differences in neuronal genomic expression. Rather, they are imposed by the gonadal steroid environment during development on an inherently female or at least bipotential brain.

The general evidence for the process of the sexual differentiation of the brain can best be summarized by a consideration of two of the most thoroughly studied sexually dimorphic functional processes. Adult male and female rats differ in terms of the regulation of LH secretion from the pituitary and in their expression of feminine sexual behavior as measured by the lordosis reflex (*1,2,4,9*). Female rats have the capacity to secrete LH cyclically, which is necessary for ovulation to occur, but the genetic male, even though castrated as an adult, does not show this surge of LH in response to the facilitatory feedback action of ovarian steroids. Moreover, under the activational effects of ovarian estradiol and progesterone, the female rat becomes sexually receptive and will readily display the lordosis reflex in response to the stimulus of mounting. The male, even though castrated as an adult and primed with the same hormonal regimen, only rarely exhibits the lordosis reflex.

The fact that these sex differences in brain function are not dependent on neuronal genomic activity directly is established by two basic observations. If one administers exogenous steroids [testosterone propionate (TP) has been used most extensively] within the first week of postnatal life, when these animals are adult the male treated with exogenous TP is still neuroendocrinologically masculine, but so is the female. This "androgenized" female does not ovulate, does not show positive feedback to gonadal steroids, and exhibits the lordosis reflex at a low rate more typical of that of the genetic male. In contrast, the opposite is seen following castration in early postnatal life. Although removal of the ovaries from the neonate is without major effect on neuroendocrine status in adulthood in the female, if the testes are removed prior to the third postnatal day of life, then this male when adult exhibits neuroendocrine characteristics typical of the female including both the ability to release a surge of LH cyclically and to exhibit feminine levels of lordosis behavior (*1,2,4,9*).

Masculine sexual behavior, although often less dramatically sexually dimorphic in the rat (*18*), is another example of a brain function which undergoes sexual differentiation (*19*). This list also includes aggressive behavior, social, play, and open field behaviors, territorial marking, urination posturing, the regulation of food intake, taste preference, learning behavior or performance, cognitive function and enzymatic activity within the hypothalamus and even the liver (*1–4,9*). Moreover, this concept applies to many species including the rat, mouse, hamster, guinea pig, gerbil, sheep, pig, and primate. It must be emphasized, however, that not all the same functions are sexually dimorphic in all species. Nevertheless, the process of the sexual differentiation of the brain is a general phenomenon both in terms of the species and functions to which it applies. It is also important to stress that the sexual differentiation of several brain functions in one species can occur independently and may have distinct temporal patterns of differentiation, different hormonal sensitivities and specific neural sites of hormone action (*19,20*).

Given the rather general importance of sexual differentiation to reproduction and even to neurobiology, it is somewhat surprising to realize that until recently essentially very little was known about the possible mechanisms of the organizational action of steroids. Since many of these sexually dimorphic functions are dependent on the activational effects of the same hormones, one could suggest that early exposure to steroids alters permanently the response of neurons to subsequent steroid exposure. However, studies of possible sex differences in steroid uptake have not provided consistent data (*21*). Although sex differences in steroid uptake (*22*) or in chromatin binding (*23*) may indeed exist, it is still not known whether this is a cause of, or an effect of, sexual differentiation. Similarly, there are a number of sex differences in neurotransmitter activity (*24–27*); do these cause or result from sexual differentiation? Finally, the precise site of steroid action is still not fully identified, although the preoptic area (POA) was clearly implicated as one such site (*19*).

Over the past decade this situation has changed dramatically. Marked structural sex differences have been identified in the brain and spinal cord and these have been shown to undergo sexual differentiation. These structural sex differences, therefore, may underlie the known functional sex differences, and provide valuable

model systems to approach the study of the mechanism of the organizational action of gonadal steroids.

Although a number of structural sex differences were known beforehand (*2*), several more recent studies were especially influential. In 1973, Raisman and Field (*28*) reported the existence of a statistically significant sex difference in the pattern of synaptic organization in the POA at the ultrastructural level. These investigators also demonstrated the importance of the steroid environment postnatally for the establishment of this sex difference. This was soon followed by the identification of dramatic sex differences at the level of nuclear organization in the songbird brain (*29*). The jump from structural sex differences seen only with the electron microscope to those that can be seen with the unaided eye, was completed with the identification of the sexually dimorphic nucleus of the preoptic area (SDN-POA) of the rat (Figs. 1*A* and 1*B*) which shall be described in detail below. However, the list of structural sex differences in the central nervous system continues to grow and currently includes sex differences in the synaptic organization in the POA (*28,30*), arcuate (*31*), amygdaloid (*32*), and suprachiasmatic (*33*) nuclei and the lateral septum (*34*), and in regional nuclear volume, e.g., the ventral medial hypothalamic nucleus (*35*), the bed nucleus of the stria terminalis (*36*), the anteroventral periventricular nucleus (*37*), as well as the SDN-POA. Structural sex differences have been reported in the songbird (*29*), rat (*28,31,35*), gerbil (*38*), guinea pig (*36*), hamster (*30*), ferret (*39*), and the human (*40,41*) brain. It is likely that these lists will continue to grow as more species are studied, as more brain regions are analyzed, and as such analyses progress to the level of the neurochemical specificity of individual neurons or neuronal groups.

The Sexually Dimorphic Nucleus of the Preoptic Area

The SDN-POA is an area of increased neuronal density which is approximately five times larger in volume in the male than in the female rat (*42*). Importantly, there is no sex difference in terms of the number of neurons per unit area within this nucleus (*43,44*). Thus, the SDN-POA of the male includes a larger number of

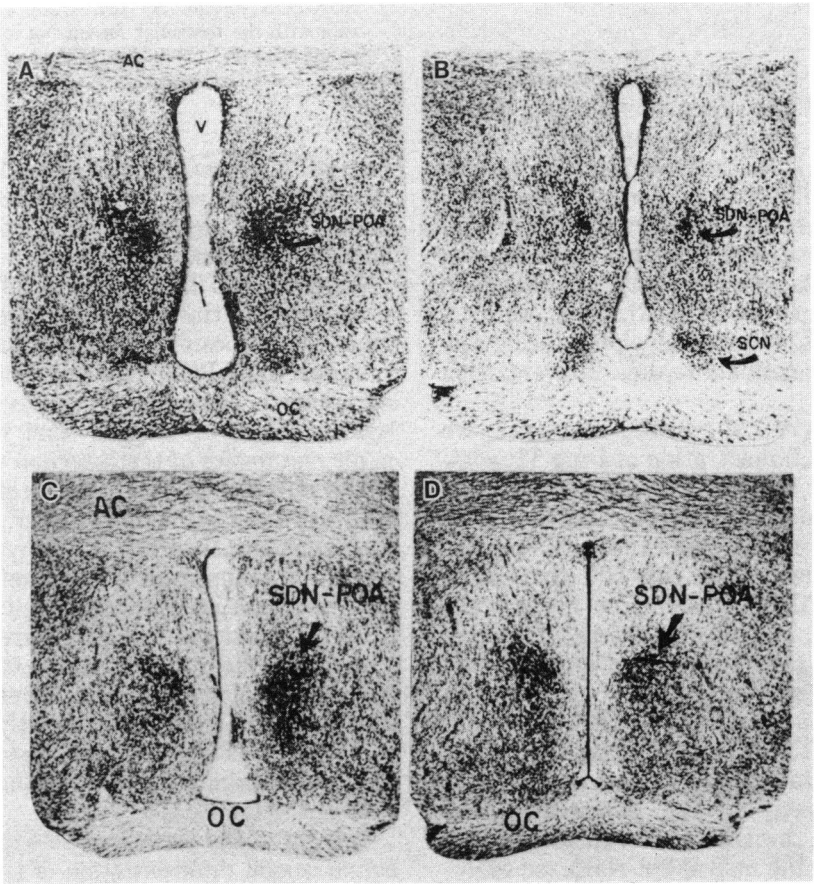

FIGURE 1. Representative coronal sections through the center of the SDN-POA of four groups of rats. The general size of the SDN-POA of the male (*A*) and female (*B*) of the adult rat is compared to that of the genetic female which was exposed from embryonic day 16 through postnatal day 10 to daily injections of testosterone propionate (*C*) or diethylstilbestrol (*D*). All at the same magnification. Abbreviations: AC, anterior commissure; OC, optic chiasm; V, third ventricle. From Gorski (*93*).

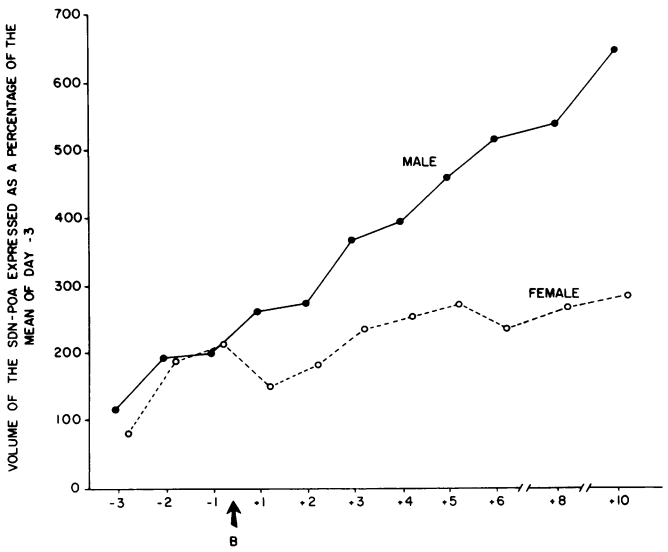

FIGURE 2. Development of the sex difference in the volume of the SDN-POA related to the day of birth (B). SDN-POA volume is expressed as a percentage of the mean combined volume of males and females sacrificed on embryonic day 20 [3 days (-3) before expected birth]. Data from Jacobson et al. (*45*); reproduced from Gorski (*94*).

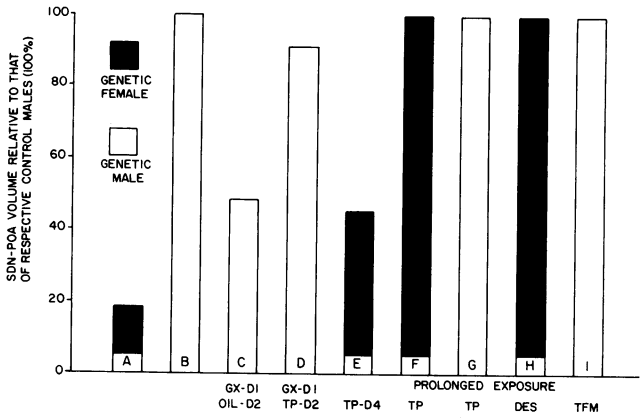

FIGURE 3. Influence of the gonadal hormone environment perinatally on SDN-POA volume in adult rats. SDN-POA volume is expressed as a percentage of the volume of this nucleus in control males from several independent studies: (*A,B*) the sex difference in SDN-POA volume; (*C*) males were castrated on postnatal day 1 (GX-D1) (*42*); (*D*) similarly treated males were then injected with 100 μg TP on postnatal day 2 (TP-D2) (*46*); (*E*) females were injected with 1 mg TP on day 4 (TP-D4) (*42*). Although daily exposure to TP from embryonic day 16 through postnatal day 10 has no effect on SDN-POA volume in males (*G*), this treatment sex-reverses nuclear volume in females (*F*) (*47*). Similarly prolonged exposure to diethylstilbestrol (DES) also sex-reverses SDN-POA volume (*H*) (*48*). Group I indicates that genetic male rats with the testicular feminizing mutation (TFM) have a masculine SDN-POA (*60*). See individual studies for statistical analyses. From Gorski (*4*).

neurons. Two questions were immediately raised by the discovery of the SDN-POA: When does the sex difference develop, and, Is it sensitive to hormones?

The SDN-POA is first statistically different in volume on day one of postnatal life (*45*). In the male, the SDN-POA continues to increase in volume over the course of the first 7 to 10 days of postnatal life, while in the female, there is essentially no statistically significant change in volume over this time period (Fig. 2). Thus, the sex difference in SDN-POA volume develops during the critical period for the functional sexual differentiation of the brain.

In terms of the influence of gonadal hormones, we demonstrated that the administration of 1 mg TP postnatally to the female rat significantly increases the volume of the SDN-POA attained in adulthood (*42*) (Fig. 3). Moreover, following castration of the male on postnatal day 1, SDN volume reaches only 50% that of the normal level (*42*). SDN-POA volume can be fully restored, however, by the injection of 100 μg TP on day 2, 24 hr after castration (*46*). Subsequently, we discovered that it is possible to sex-reverse SDN-POA volume completely by prolonged exposure to TP perinatally. We administered 2 mg TP daily to pregnant rats from day 16 postfertilization through parturition and then 0.1 mg TP to the pups directly through postnatal day 10 (*47*). As seen in Figure 3, this treatment had no influence on SDN-POA volume in the males, but rendered SDN-POA volume in females equivalent to that of males.

Although such prolonged hormonal exposure does not rule out a possible contribution of a genomic factor in normal development, it clearly demonstrates that hormones alone can determine SDN-POA volume and the number of neurons which comprise this nucleus. Thus, the SDN-POA can be viewed as a morphological signature of the organizational action of steroids on the developing brain.

Figure 3 also illustrates the influence of the similarly prolonged treatment perinatally with diethylstilbestrol. Note that exposure to this synthetic estrogen also sex reverses SDN-POA volume in the female (*48*). This observation is consistent with several lines of evidence which collectively suggest that it is actually an estrogenic metabolite of testosterone which affects the masculine differentiation of the brain. The enzyme aromatase is present in the brain (*49*). Estradiol benzoate (EB) is more potent than TP (*50*). Dihydrotestosterone which cannot be aromatized is very ineffective (*51,52*). Inhibitors of aromatization (*53,54*) and anti-estrogens (*55,56*) prevent functional sexual differentiation in the male. Finally, in the rat with the testicular feminizing mutation (*57*) which produces a dramatic deficit in androgen receptors (*58*) without apparently affecting the normal complement of estradiol receptors (*59*), SDN-POA volume is comparable to that of the normal male, not to that of the female (*60*).

In light of the presumed role of estrogen in the masculine sexual differentiation of the brain, the observation that in both neonatal males and females plasma estrogen titers are high (*61,62*) has presented a problem which is still not completely resolved. It is generally accepted that α-fetoprotein, which binds estradiol in the rat (*63–65*), serves to protect the brain from exposure

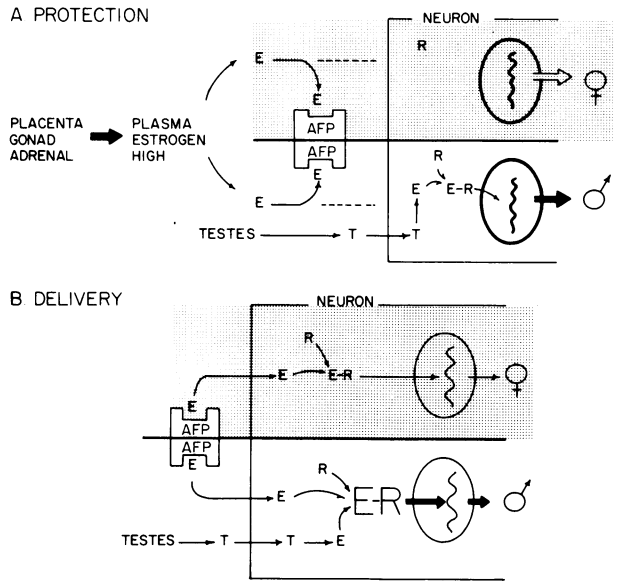

FIGURE 4. Two models of the possible role of α-fetoprotein (AFP) during the perinatal period of the sexual differentiation of the rat brain. (*A*) AFP serves to "protect" neurons of both the female (shaded) and male (clear) from exposure to plasma estradiol (*E*). (*B*) AFP serves to "deliver" this hormone to the female brain for its normal development. In both models, E derived from the intraneuronal aromatization of testosterone (T) is responsible for the masculinization of the male brain. Other abbreviations: E-R, estrogen-receptor complex; R, estrogen receptor. From Gorski (*2*).

to systemic estradiol in both sexes (Fig. 4*A*). However, since testosterone is not bound by α-fetoprotein, this testicular product can enter neurons of males where it is aromatized to an active estrogenic form. However, Dohler and Hancke (*66*) have argued that exposure to some estrogen is required for the normal development and/or differentiation of the female brain (Fig. 4*B*). Indeed, treatment postnatally with the antiestrogen tamoxifen, reduces SDN-POA volume in males as expected, but also does so in females (*67*). Moreoever, the results of the *in vitro* studies of Toran-Allerand (*68*) suggest that estradiol may be a prerequisite for the growth of neuronal processes. Finally, it is conceivable that α-fetoprotein, which she has localized within neurons, may play a role in internalizing estradiol or perhaps a more direct role of its own (*68*). Although these questions need to be resolved, at this point, we conclude that gonadal steroids, most likely estradiol, in some way determine the number of neurons which form the SDN-POA.

Mechanisms of Steroid Action

Figure 5 schematically illustrates six possible and nonexclusive mechanisms by which gonadal steroids might influence the number of neurons which eventually comprise the SDN-POA. Testicular hormones could stimulate or prolong neurogenesis, influence the process of migration of SDN-POA neurons from their origin to the general region of the nucleus, promote the aggregation or coalescence of neurons into the nucleus, prevent neuronal death (or promote survival) during the process of migration or during the establishment of critical connections and/or influence the specification of SDN-POA neurons in terms of their morphological or chemical nature.

Initially it appeared unlikely that an effect of gonadal steroids on neurogenesis was involved in the generation of the SDN-POA. This view was based on the consistent observation that the neurons of the medial POA are essentially born (i.e., have become postmitotic) by embryonic day 16 (*69–71*); yet SDN-POA volume can be modified postnatally more than a week after the neurons become postmitotic. Nevertheless, we attempted to verify the birth date of SDN-POA neurons specifically since the previous studies were conducted before the existence of this nucleus was recognized. As illustrated in Figure 6, the results of this study essentially confirmed the data in the literature in terms of the POA in general, but also revealed that the genesis of SDN-POA neurons is specifically and quite significantly prolonged temporally (*44*). In fact, by administering tritiated thymidine on embryonic day 18, we specifically and permanently label a subcomponent of the neurons of the SDN-POA, a late arising subcomponent (compare Figs. 7*A* and 7*B*). Moreover, analysis of these data revealed two statistically significant sex differences in "apparent neurogenesis." The labeling index following exposure to tritiated thymidine on embryonic day 14 is greater in the SDN-POA of the female, and on embryonic day 17 is greater in the SDN-POA of the male (Fig. 6).

The interpretation of these results is difficult principally because the brains of these animals were not evaluated until postnatal day 30. Thus, sex differences in any one of the possible mechanisms illustrated in Figure 5, could affect the labeling index. However, a putative neuronal mitogenic effect of gonadal steroids, even if minor, could well influence the final number of neurons in the SDN-POA and would represent a very interesting biological observation, but currently this unanswered question can best be challenged *in vitro*.

The fact that the late arising subcomponent of SDN-POA neurons can be specifically labeled with tritiated thymidine on embryonic day 18, has permitted us to identify the origin of these neurons, their presumed pathway of migration and to attempt to detect any sex or hormone-dependent differences in these processes (*72*). As illustrated in Figure 7*C–F*, the neuroblasts which form these late arising neurons are located in the lateral walls of the third ventricle. The labeled cells then appear to migrate to the base of the ventricle and into the surrounding neural parenchyma. The neurons then appear to migrate upward and laterally and coalesce in the region of the SDN-POA itself. Interestingly, the labeled cells between the base of the brain and the SDN-POA clearly diminish in number over time.

Although statistical analysis of the change in location over time of labeled cells based on a two-dimensional grid system establishes that migration occurs (*71*), we

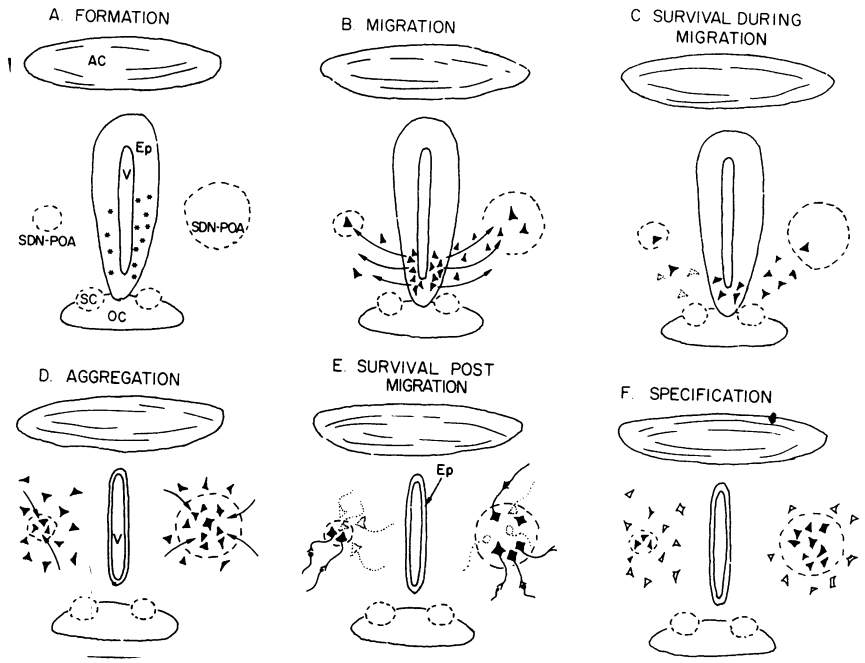

FIGURE 5. Highly schematic representation of six theoretical, and not mutually exclusive, mechanisms by which estradiol might influence the number of neurons which ultimately reside in the SDN-POA. The right side of each panel represents the putative action of gonadal steroids. Abbreviations: AC, anterior commissure; Ep, ependymal lining of the third ventricle (V); OC, optic chiasm. From Gorski (*93*).

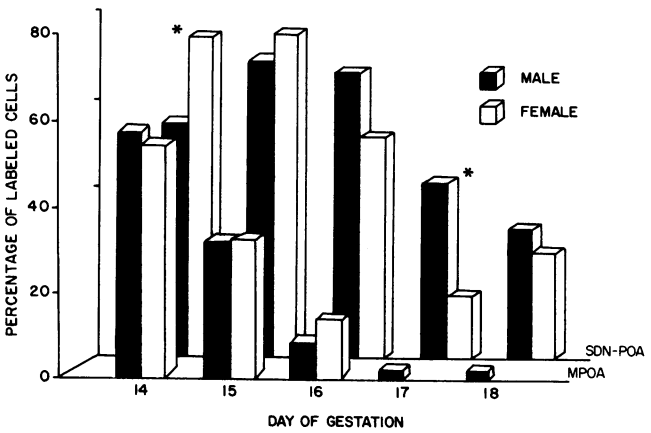

FIGURE 6. The pattern of neurogenesis for the SDN-POA and a region of the medial preoptic area (MPOA) just lateral to the former. Tritiated thymidine was injected to pregnant rats on the indicated day of gestation and the offspring sacrificed on postnatal day 30. Asterisks indicate a statistical sex difference in the percentage of labeled cells within the SDN-POA. Data from Jacobson and Gorski (*44*). Reprinted from Gorski (*93*).

have not been able to detect any significant sex or hormone-dependent difference (i.e., by comparing male and female, or intact males and those castrated on postnatal day 1). It is possible that these late arising neurons of the SDN-POA are atypical and are not, in fact, sensitive to hormones. However, we have recently obtained preliminary data on the number of cells of the SDN-POA labeled with tritiated thymidine on embryonic day 18 in control males and females and females in which SDN-POA volume has been sex reversed by treatment with TP beginning either on embryonic day 16 or 20 and continuing through postnatal day 10. In this case, the number of labeled cells in the males and both groups of sex reversed females was identical and more than double the number of labeled cells in the control females. Thus, these late-arising neurons are sensitive to steroids and may indeed be representative of the general population of SDN-POA neurons. The possibility of a sex or hormonal effect on the migration of SDN-POA neurons requires a more precise analysis before being definitively refuted.

At present it is not possible to study the role sex differences in the aggregation process might play in the formation of the SDN-POA, nor whether steroid hormones modify the morphological development (e.g., staining characteristics) of the neurons in this region. It is generally accepted, however, that the major action of steroids involves the survival of postmitotic neurons. Although this concept has not yet been proven applicable to SDN-POA neurons, it does appear to apply to another structural sex dimorphism: the spinal nucleus of the bulbocavernosus (*73*). Several observations support this view in the case of the SDN-POA as well.

First, the most dramatic changes in SDN-POA volume do occur postnatally, when the neurons of this nucleus are clearly postmitotic. Second, it is now well established that during development there is a phase of neuronal death which is quite extensive; 50–75% of the neurons in a given structure may die during development (*74–76*). During the course of development neurons migrate to their final destination, perhaps by being

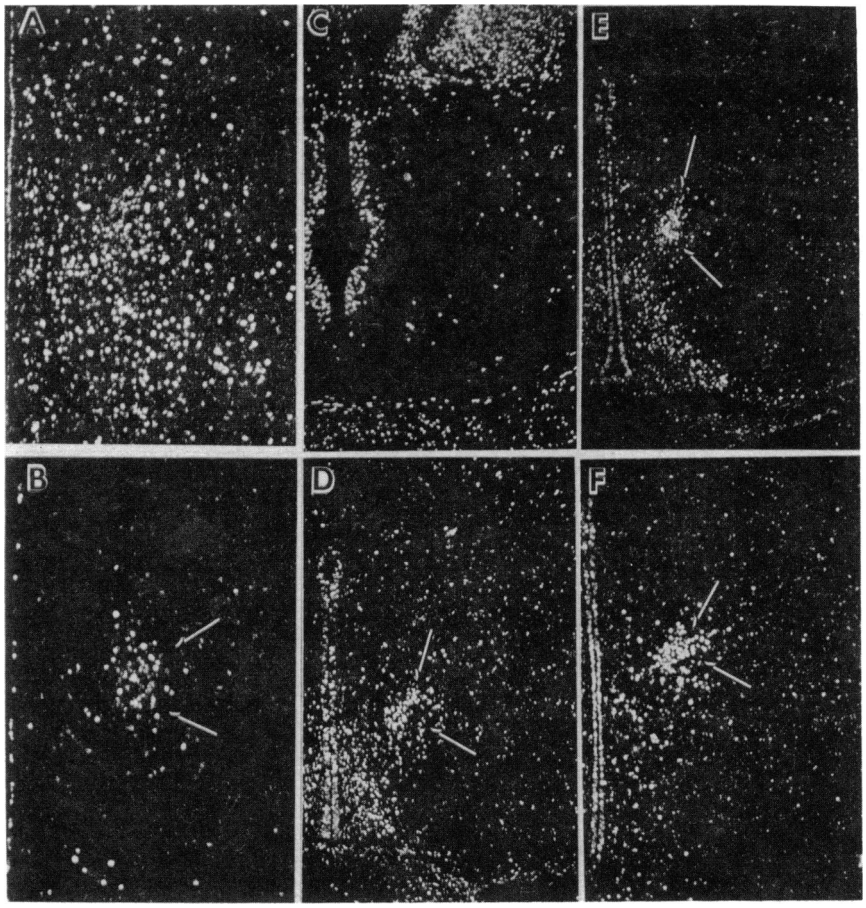

FIGURE 7. Dark-field photomicrographs of coronal sections through the SDN-POA (arrows) of rats exposed to tritiated thymidine in utero. Comparison of *A* and *B* (both at the same magnification) demonstrates the specific prolongation of neurogenesis of the SDN-POA. Pregnant rats were injected with tritiated thymidine on embryonic day 15 (*A*) or 18 (*B*) and the pups sacrificed on postnatal day 30. Modified from Jacobson and Gorski (*44*). (*C-F*) (all at the same magnification) illustrate the change in position of labeled cells over perinatal development. All rats were exposed to tritiated thymidine on embryonic day 18 via an injection to their mothers, but sacrificed at 18 days + 2 hr (*C*), 22 days (*D*), 26 days (*E*), or 32 days (*F*) post-fertilization. Modified from Jacobson et al. (*72*). Reprinted from Gorski (*95*).

guided by glial elements (*77*) or chemical factors, then these neurons extend processes and make connections with their targets. During this process the neurons appear to be maintained by proteinaceous growth factors, the extracellular matrix, and possible humoral factors. When anatomical connections are made, the neurons appear to change in some unknown way and many abruptly die. It is assumed that the target cells produce another neuronotrophic substance which is now essential for survival. This substance, which is presumed to be produced only in limited amounts, is transported retrogradely to the neuronal soma, and there acts to permit survival and differentiation. Neurons which reach this stage of dependence on their targets' neuronotrophic substance(s) before they make appropriate connections, or those which simply do not attain enough or fail to respond to it, die.

Finally, the work of Toran-Allerand (*68*) has established that for estrogen-responsive hypothalamic neurons of the mouse, exposure to estrogen stimulates and is essential for neurite outgrowth *in vitro*. If this also applies *in vivo*, it can be suggested that under the stimulus of estradiol derived from the aromatization of testicular testosterone, the growth of processes from SDN-POA neurons is promoted so that more of these neurons make their appropriate connections and survive. In the female, however, without the stimulus provided by estrogen, fewer neurons make appropriate connections either anatomically or temporally, so many more neurons die.

In this context, therefore, estrogen appears to lead to the sex difference in the SDN-POA by virtue of a neurite outgrowth promoting action. Analysis of Figure 7 suggests an additional possibility. It is clear that the number of tritiated thymidine labeled cells which are located between the base of the brain and the SDN-POA diminishes during the first week of life. Although these neurons may merely migrate, either to the SDN-POA or elsewhere, it is possible that cell death also occurs during this period of migration. If this is true,

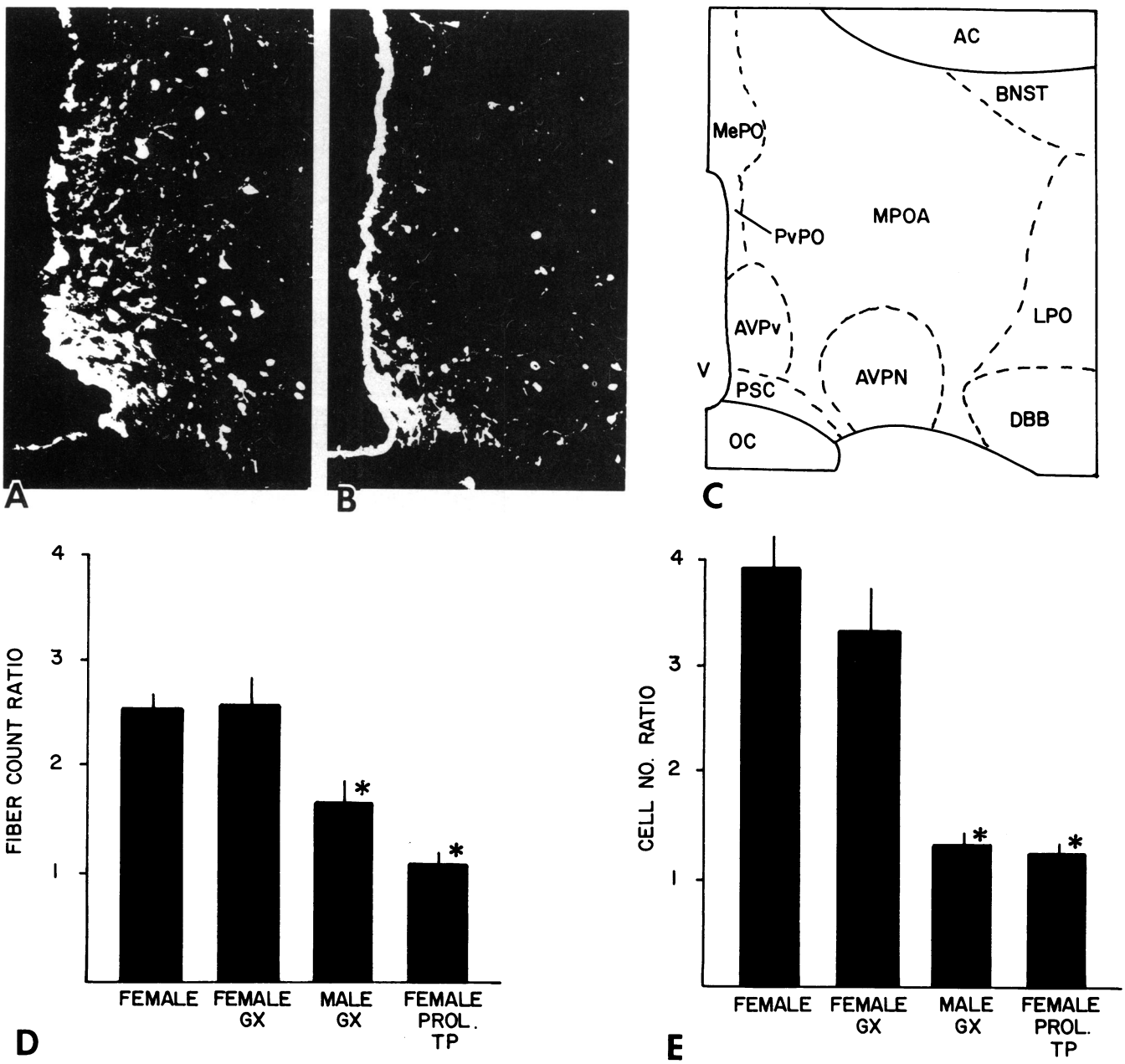

FIGURE 8. Sex difference in the distribution of putative dopaminergic cells and fibers in the anteroventral periventricular nucleus (AVPV) and the influence of prolonged exposure perinatally to TP on these distributions. (A,B) fluorescence photomicrographs of tyrosine hydroxylase immunoreactive cells and fibers of colchicine-treated female (*A*) and male (*B*) rats; from Simerly et al. (*37*). (*C*) General location of the AVPV. The effect of gonadectomy (GX) in male and female rats, and of prolonged exposure to TP (prol. TP) perinatally in females is shown as an index of fiber density (*D*) and the number of immunostained cell bodies (*E*). Both are expressed as ratios between treatment groups and an intact reference male rat. Asterisks: significantly different from intact female. Data from Simerly et al. (*78*). Reprinted from Gorski (*4*).

then estrogen may be directly neuronotrophic for these migrating neurons, maintaining their survival until they establish their connections.

It is obvious that further studies are required before the mechanism(s) of steroid action on the developing SDN-POA is fully elucidated. Nevertheless, the existence of structural sex differences in the central nervous system, such as the SDN-POA, should permit us to establish whether estrogen is a neural mitogen, a neurite outgrowth promoter and/or a neuronotrophic substance, and eventually to unravel these actions at the molecular level.

Although it is tempting to assume that the mechanism(s) of steroid action that apply to the development of the SDN-POA will be of general applicability, such an assumption is most likely an oversimplification. For example, there is experimental evidence that gonadal steroids can also decrease the number in a specific nu-

cleus of chemically defined neurons (*78*). Bleier and her colleagues (*79*) have reported that a nucleus, which has been implicated in the control of ovulation (*80*), is larger in the female rat. Although these authors have labeled this the medial preoptic nucleus, we prefer a different nomenclature and have labeled it the anteroventral periventricular nucleus (AVPV) (*37*).

Recently we have evaluated the AVPV in terms of immunohistochemistry with antibodies directed against tyrosine hydroxylase (TH) and dopamine beta-hydroxylase (DBH). Immunohistochemical staining against DBH is sparse and not sexually dimorphic (*37*). However, TH-immunoreactive fibers and somata are present in the AVPV, and there is a quantitative sex difference (Fig. 8). Because of their TH immunoreactivity and the lack of DBH immunoreactivity, we consider these elements dopaminergic. As shown in Figure 8E, there are approximately four times as many dopaminergic neurons in the AVPV in the female and importantly this sex difference is dependent on the hormonal environment perinatally (*78*). Exposure to TP markedly reduces the number of TH immunoreactive neurons. From these results it can be suggested that in this system steroids either promote neuronal death or the neurochemical specification of the dopaminergic neurons of the AVPV. The smaller size of the AVPV of the male supports the first interpretation and suggests that steroid action perinatally is necessarily trophic for all neuronal systems.

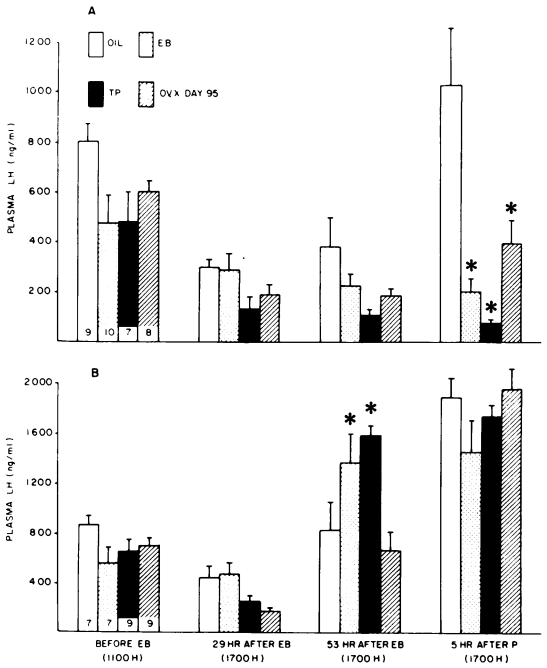

FIGURE 9. Influence of endogenous ovarian factors or exogenous steroids on facilitatory steroid feedback in female rats. Plasma samples were obtained before estradiol benzoate (EB) injection, 29 and 53 hr later, and again 5 hr after progesterone (P) injection in rats ovariectomized (OVX) at 95 days of age, or at 30 days and then given daily treatment with oil, 0.5 μg EB or 100 μg TP for 60 days: (*A*) lightly androgenized rats; (*B*) normal female rats. Asterisk: significantly different from rats given daily injections of oil. Note change of scale between *A* and *B*. From Harlan and Gorski (*92*).

Hormonal Action Beyond the Perinatal Period

The present discussion has focused on the permanent or organizational effects of gonadal steroids which take place during development perinatally and their morphological bases. Are such effects actually limited to the perinatal period? The answer to this question is clearly no. It has been shown, for example, that estradiol stimulates synaptogenesis peripuberally (*81*) and after deafferentation in the young adult (*82*) and that testosterone promotes regeneration of the transected hypoglossal nerve in the adult rat (*83*). In the gerbil, the volume of a sexually dimorphic area of the hypothalamus, in contrast to that of the SDN-POA in the rat, is sensitive to the hormonal environment in the adult (*38*). Finally, in the songbird, there are seasonal variations in neuronal structure which are hormone dependent (*84–86*). Thus, gonadal steroids do alter neuronal structure even in adulthood.

There is also evidence for permanent effects of the peripubertal hormone environment in terms of reproductive function. Although the exposure of the neonatal female rat to TP suppresses ovulation, this effect is dose dependent (*87*); in fact, an interesting syndrome follows the exposure of the female rat to a low dose (10μg) of TP on postnatal day 5. After puberty, these animals exhibit regular ovulatory estrous cycles, but around 65 days of age on the average, they become anovulatory and exhibit persistent vaginal estrus. Because the appearance of the anovulatory condition is temporally delayed in these lightly androgenized rats in comparison to the fully androgenized rats which are anovulatory from the time of vaginal opening, we have called this the delayed anovulation syndrome (DAS) (*87*).

Kikuyama and Kawashima (*88*) and later Arai (*89*) reported an interesting observation directly relevant to this question. If lightly androgenized rats are ovariectomized prior to puberty, ovulation will occur in ovarian tissue grafted in these animals at an age well beyond the age at which intact animals have already become anovulatory. It would appear from these results that the presence of the ovaries contributes to the loss of ovulation. As shown in Figure 9, we have confirmed this observation using the estrogen-progesterone induced surge of LH as the index of hypothalamic function (*90*). Lightly androgenized rats ovariectomized at 90 days of age do not exhibit an LH surge following the sequential injections of estrogen and progesterone, but at the same age, lightly androgenized rats which were ovariectomized at day 30 do. Moreover, daily injections of 0.5 μg EB or 100 μg TP for 60 days following ovariectomy on day 30, block this positive feedback response. Thus, in these lightly androgenized rats, some ovarian factor,

Table 1. Effect of estrogen treatment at various ages on the onset of puberty as measured by vaginal opening (VO), and the onset of persistent vaginal estrus (PVE).

Treatment	N	Day of VO	Day of onset of PVE	Total days cycled[a]
EB prepuberally (0.05 μg/day, age 26–30 days)				
Control + oil	28	39.5 ± 0.3[b]*	NA[c]	NA
+ EB	13	31.4 ± 0.2*	NA	NA
Lightly androgenized				
+ oil	16	37.9 ± 0.5	72.4 ± 3.5	35.2 ± 3.8
+ EB	24	33.7 ± 0.4*	98.0 ± 5.1*	63.6 ± 5.3
No treatment	17	37.5 ± 0.4	67.4 ± 4.2	30.2 ± 4.1
EB peripuberally (0.05 μg/day, age 33–37 days				
Control + EB	16	37.0 ± 0.3	NA	NA
Lightly androgenized				
+ oil	13	37.9 ± 0.7	78.0 ± 4.1	40.5 ± 4.3
+ EB	28	37.4 ± 0.3	63.1 ± 2.6	25.4 ± 2.6*†

[a] The number of days cycled was obtained by subtracting the day of VO from the first day of PVE.
[b] All values are mean number of days ± SEM.
[c] NA indicates that these animals did not enter PVE during the ages studied.
* Significantly different ($p < 0.01$) from all other groups.
† Significantly different ($p < 0.01$) from matched oil-treated controls.

presumably steroids, acts peripuberally and advances or promotes ovulatory failure.

In more recent studies we have investigated the influence of brief exposures to low doses of EB. Ramirez and Sawyer (*91*) had demonstrated that the prepubertal injection of EB can accelerate puberty. We argued that similar treatment in the lightly androgenized rat would also accelerate puberty and perhaps hasten the onset of the DAS. When EB was given peripuberally, i.e., on days 26 to 30, vaginal opening was significantly accelerated, but in contrast to our expectations the onset of persistent vaginal estrus was markedly delayed, and thus, the period of estrous cycling significantly prolonged (Table 1) (*92*). In contrast, when the same dose of EB was given peripuberally, i.e., on days 33 to 37, although there was no effect on vaginal opening, the onset of persistent vaginal estrus was significantly advanced as predicted from the data shown in Figure 8.

These studies collectively demonstrate an interaction between steroid action perinatally and the peripubertal hormonal environment, and also emphasize that the action of steroids is clearly dependent on the precise temporal window of exposure.

Conclusions

The sexual differentiation of the brain is an important physiological process which permanently establishes sex differences in the functional potential of the brain to regulate the reproductive process. It is assumed that structural sex differences in the central nervous system may underlie some or all of the numerous functional sex differences that exist and it is likely that the mechanism of the morphological actions of gonadal steroids will be further elucidated in the foreseeable future, perhaps at the molecular level. Such information will hopefully contribute to our understanding of the toxicological alterations in the reproductive system which occur during development in response to xenobiotics, environmental chemical factors, and as unwanted side effects of drug administration. The fact that exposure to steroids even beyond puberty can have morphological and permanent functional effects suggests that the brain can be sensitive to such factors throughout life.

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