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108. This work was supported by an Alfred T. Sloan Foundation fellowship (to N.J.M.) and by grant HD 13587 from the National Science Foundation.

Neural Gonadal Steroid Actions

Bruce S. McEwen

To understand sex differences in behavior, we must understand the mechanisms that control these behaviors in adult life as well as the factors and mechanisms involved in their development. Because of the considerable degree to which environmental factors and learning play a role in behavior in our own species, investigators have turned to other species to study stereotyped behaviors as well as the underlying brain mechanisms. This has been a satisfactory approach for the study of behaviors regulated by hormones.

Goy has classified the sexually differentiated aspects of behavior into three categories: type 1, those that undergo differentiation under the influence of hormones and are also activated by hormones; and type 2, those that undergo differentiation independently of the influence of hormones but are activated by hormones; and type 3, those that are influenced by hormones during differentiation but are not activated by hormones (*1*).

Summary. Neurons sensitive to gonadal steroids are located strategically within neural circuits that mediate behaviors broadly related to the reproductive process. Some neuronal events and properties are regulated by these hormones. Variability in the occurrence and distribution of particular neural hormonal sensitivities across species may be related to variations in the hormonal requirements for sexual differentiation and for activation of reproductive behaviors.

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For the most part, this article focuses on type 1 mechanisms, which include many of the components of reproductive behavior, broadly defined to include courtship, definition and defense of territory, and mating. I review what has been learned about the cellular mechanisms by which hormones activate behavior in a few species. I then consider some of the ways in which this information may be relevant to our understanding of the sexually differentiated features of the brain and behavior across species.

Location of Neurons Sensitive to Gonadal Steroids in the Brain

Studies of hormone action on the brain at the cellular level have been facilitated by the localization of hormone-sensitive cell groups with biochemical and autoradiographic techniques. Estrogen, androgen, and, most recently, progestin receptors have been characterized and mapped within the brain (*2*). Much of this work has been done on the rodent brain, but we also have a good idea about receptor systems in brains of other mammals and of members of other vertebrate classes, as described below.

The map of estrogen-sensitive cells in the rat brain obtained by autoradiography (*3*) reveals clusters of estrophilic cells in the hypophysiotropic area as well as the corticomedial amygdala. The pattern of in vivo uptake and cell nuclear retention (*5*) of ^{3}H-labeled estradiol reflects this distribution (Fig. 1). Fewer and lesser labeled cells are found in regions such as the mesencephalic central gray and hippocampus (*3*).

Androgen-sensitive neurons are difficult to map owing to the fact that testosterone is extensively converted to estradiol (Fig. 1) as well as to 5α-dihydrotestosterone (DHT) in the brain (Fig. 2). Estradiol and DHT attach to estrogen and androgen receptors, respectively (Figs. 1 and 2). A problem in using DHT to study androgen receptors is that DHT is extensively metabolized when given systemically (*4*). However, enough DHT reaches the brain so that it is possible to obtain information about the distribution of androgen receptor sites; such studies have revealed a pattern of androgen-sensitive neurons (*5*, *6*) which overlaps to some extent with that of the estrogen-

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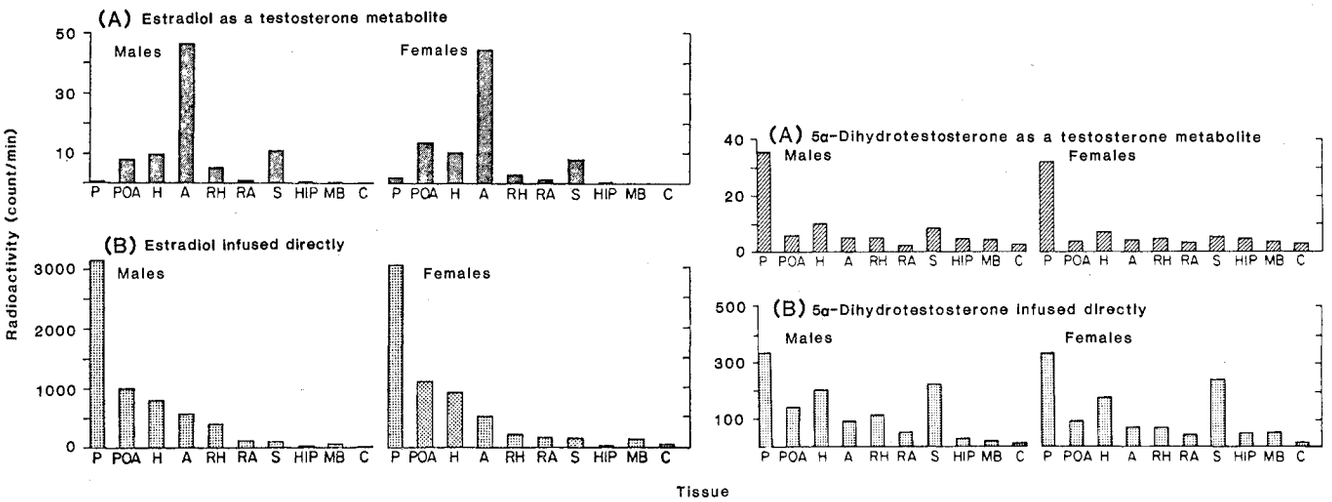

Fig. 1 (left). (A) Radioactivity is identified as 17β-estradiol present in brain cell nuclear fractions 2 hours after administration of ^{3}H-labeled testosterone (5.7 μg/kg) to castrated adrenalectomized adult male and female rats. (B) Radioactivity in brain cell nuclear fractions 2 hours after administration of ^{3}H-labeled 17β-estradiol (2.7 μg/kg) to castrated adrenalectomized adult male and female rats. The values are means of four determinations for each sex, Abbreviations: *P*, whole pituitary; *POA*, preoptic area; *H*, basomedial hypothalamus; *A*, corticomedial amygdala; *RH*, rest of hypothalamus; *RA*, rest of amygdala; *S*, septum; *HIP*, hippocampus; *MB*, midbrain-central gray; and *C*, parietal cerebral cortex. [Reprinted from (*5*); courtesy of *Endocrinology*] Fig. 2. (right). (A) Radioactivity is identified as dihydrotestosterone present in brain cell nuclear fractions 2 hours after administration of ^{3}H-labeled testosterone (5.7 μg/kg) to castrated adrenalectomized adult male and female rats. (B) Levels of radioactivity in cell nuclear fractions 2 hours after administration of ^{3}H-labeled dihydrotestosterone (2 to 4 μg/kg) to castrated adrenalectomized adult male and female rats. Values are the means of two determinations for each sex. Abbreviations are identical to those listed in the legend to Fig. 1. [Reprinted from (*5*); courtesy of *Endocrinology*]

sensitive neurons (Fig. 2). It is possible that estrogen and androgen receptors exist in the same neurons in some of these sites.

Progestin receptors exist in some of the estrophilic nerve cells of the brain (*7*). There is overlap in the autoradiographic maps of estrogen- and progestin-concentrating cells of the hypophysiotropic area of the rat and guinea pig, particularly in midline and ventral structures such as the medial and periventricular preoptic area and arcuate and ventromedial nuclei (*7*). Moreover, estradiol induces progestin receptor sites (*8*) within the preoptic area and hypothalamus (Fig. 3). Induction of progestin receptors is not an invariant characteristic of estrogen-concentrating neurons—those of the amygdala fail to show progestin receptor induction by estradiol (*8*). Nor are progestin receptors found only in estrogen-concentrating regions of the brain; the brains of the rat (*8*), guinea pig (*9*), and lizard (*10*), for example, contain progestin receptors outside of the hypophysiotropic region, for example, in cerebral cortex (Fig. 3). The possible significance of this is discussed later.

Location and Function of Enzymes That Transform Gonadal Steroids

Transformation of gonadal steroids by neural tissue plays a role in the action of certain of these hormones. Perhaps the best example is the transformation (Fig. 4) of testosterone to estradiol (*11*) by aromatization. This transformation has been implicated in the rat brain for the activation of male sexual behavior (*12, 13*) and for the defeminizing aspects of testosterone action in the sexual differentiation of the brain (*14*).

Aromatizing enzymes are concentrated within certain brain regions that have estrogen receptor sites, such as the amygdala, hypothalamus, and preoptic area (*4, 11, 15*), where their actions result in the occupation of estrogen receptor sites by estrogen derived from testosterone (Fig. 1). The relative amounts of aromatizing enzyme activity differ in relation to the concentration of estrogen receptors. In some rat brain areas, for example, the amygdala, extensive estrogen receptor occupation occurs after testosterone treatment. In other areas, like the pituitary, aromatization is undetectable (*11*), and no estrogen receptor occupation is found after testosterone treatment. Recent estimates of the capacity of the estrogen receptor system to bind estradiol produced by aromatization indicate that approximately half of the neural estrogen receptor sites may never see estradiol arising from testosterone (*16*). This suggests that a subset of the estrogen-sensitive cells is capable of aromatization.

Besides aromatizing testosterone, the brain also contains an enzyme (5α-reductase) which converts testosterone to DHT (Fig. 4). Dihydrotestosterone binds to androgen receptors (*4, 5*) (Fig. 2). Testosterone itself is also able to attach to androgen receptor sites in brain and pituitary (*5*) as well as in other tissues. This raises the question of the existence of more than one class of androgen receptors: one preferring testosterone, the other DHT. This question remains unanswered (*17*). Because there is no effective inhibitor of 5α-reductase, the relative contribution of dihydrotestosterone and testosterone to androgen effects has not yet been determined. Concerning the more general question of the involvement of androgens (testosterone or dihydrotestosterone) and androgen receptors in neuroendocrine events, Krey *et al.* have convincingly demonstrated that in the rat androgen receptors may mediate the negative feedback effects of testosterone on gonadotropin secretion (*18*). These studies were made with flutamide (an androgen antagonist) and ATD (1,4,6-androstatriene-3,17-dione), an inhibitor of aromatization, as well as the androgen-insensitive, receptor-deficient mutant.

The 5α reduction of progesterone also occurs in the brain and pituitary (Fig. 4). Because 5α-reductase is responsible for 5α reduction of both testosterone and progesterone, competition by progesterone has been proposed as a mechanism by which progesterone may attenuate androgen action (*19*). The functions of 5α-dihydroprogesterone (Fig. 4), as well as of other metabolites of progesterone, have been difficult to establish

(*2*). In general, the potencies of various progesterone metabolites in their action on neuroendocrine and behavioral events is less than or at best equal to the potency of progesterone itself, a finding that does not support an obligatory intermediary role of progesterone metabolites (*2*).

Another steroid transformation occurring in the brain is the 2- or 4-hydroxylation of estradiol or estrone (Fig. 4). These steroids, termed "catechol estrogens," have been shown to interact to some degree with catecholamine enzymes [for example, catechol-*O*-methyltransferase (*20*) and tyrosine hydroxylase (*21*) and with catecholamine receptors (*22*)]. Their potencies for in vitro binding to intracellular estrogen receptor sites are three to four and four to six times less for 2-hydroxyestradiol and 4-hydroxyestradiol, respectively, compared to estradiol (*23*). Their in vivo potencies as classical estrogens with respect to activation of sexual behavior, the luteinizing hormone surge, and uterine enzyme increases are 100- and 10-fold less for 2-hydroxyestradiol and 4-hydroxyestradiol, respectively, compared to estradiol (*24*). The difference between in vitro and in vivo potencies may be explained by catabolism of catechol estrogens or by lesser penetration into the brain. Although it seems unlikely from the data that catechol estrogens mediate the classical estrogen effects on intracellular receptor sites, the fact of in situ formation of catechol estrogens in brain (*25*) makes it possible that local concentrations of these steroids may reach high enough levels to interact with some biogenic amine receptors and enzymes to alter catecholaminergic neurotransmission.

Localization of Behavioral Actions of Gonadal Steroids Within the Brain

As stated earlier, some animal behaviors are facilitated (that is, activated) by gonadal hormones. Among these hormone-dependent behaviors are ones related to mating as well as courtship and defense of territory, including vocalization. The localization of steroid-sensitive neurons by means of tritium-labeled steroids has aided immeasurably in the study of some of these behavioral actions of gonadal steroids in the brain. Two examples illustrate this.

First, the demonstration of androgen-sensitive neurons in widely separated areas of the songbird brain (*26*) was complemented by neuroanatomical studies showing that these androphilic neurons formed part of a circuit involved in vocalization, an androgen-sensitive behavior in male songbirds (*27*). These discoveries led, in turn, to the finding that some of the cell groupings of the song system are of different size in the male and female brain (*28*), and this has led further to recent studies indicating organizational effects of gonadal steroids after hatching on the morphology as well as function of the song system (*29*).

Second, the localization of estrogen-sensitive neurons within the hypophysiotropic area of the rat brain complemented studies with implants of ovarian fragments and estradiol, studies which had established this brain area as the site of estrogen action on sexual receptivity and of estrogen negative feedback on gonadotropin secretion (*2*). Such studies, together with information about aromatization of testosterone, led to estrogen implant studies which established the medial preoptic area (MPOA) as an important site for the activation of male copulatory behavior in rats (*2*, *13*). The localization of estrogen receptors by autoradiography and of estrogen effects by hormone implants also complements observations regarding the disruptive effects of MPOA lesions on male copulatory behavior (*30*) and of ventromedial hypothalamic (VMH) lesions on feminine sexual receptivity (*31*), as well as demonstrations of the effects of electrical stimulation of the MPOA on male copulatory behavior (*32*) and stimulation of the VMH on feminine sexual receptivity (*33*).

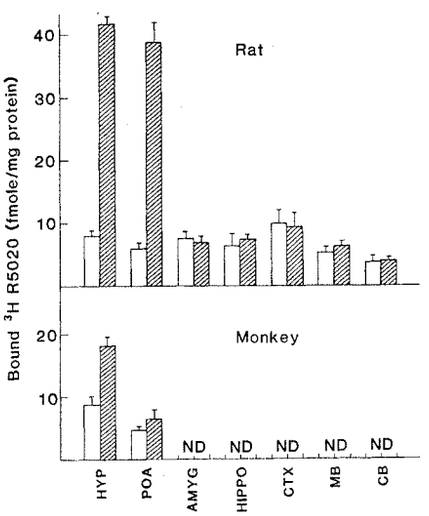

Fig. 3. Effects of estrogen treatment on the distribution of cytosol high-affinity ^{3}H-labeled R5020 binding sites in rat and monkey brain. Ovariectomized rats and monkeys were implanted subcutaneously with either Silastic capsules containing no estrogen (□) or capsules containing 17β-estradiol (▨). Cytosols from both rats and monkeys were incubated for 4 hours at 2° to 4°C with 0.4 n*M* ^{3}H-labeled R5020 in the presence and absence of 2×10^{-8}*M* unlabeled R5020. Bound steroid in the incubation mixtures was measured by Sephadex LH-20 gel filtration. High-affinity binding, defined as the difference between the results in the presence and absence of the unlabeled R5020, is expressed as the amount per milligram of cytosol protein. Results represent the means ± the standard error of four (rat) or three (monkey) observations. Abbreviations: *HYP*, hypothalamus; *POA*, preoptic area; *AMYG*, amygdala; *HIPPO*, hippocampus; *CTX*, cortex; *MB*, midbrain; and *CB*, cerebellum; *ND*, not determined. [Reprinted from (*78*); courtesy of *Endocrinology*]

Studies of the role of the VMH in feminine sexual receptivity have presented new possibilities for our understanding of the role of this brain region in sexual behavior and of the mechanism of its response to gonadal steroids. A first step in this direction involved the refinement of the technique for localizing steroid effects by implantation, in which we used ^{3}H-labeled estradiol of high specific activity in amounts about one-thousandth those of estrogen used in earlier work (*34*). This accomplished two objectives: the lesser amount in the cannula (10 nanograms of estradiol compared to 10 micrograms in earlier studies) reduced leakage, as judged by such means as uterine weight increases (*35*), and the ^{3}H label permitted assessment of spread of hormone (*34*). More than half of the ovariectomized rats displayed receptivity 3 days after bilateral implants of tritiated estradiol (*34*), and those that responded had bilateral placements of the cannulae tips near the lateral edge of the ventromedial nucleus (*34*, *35*), a region containing estrophilic neurons (*3*). Total estrogen receptor occupation in the whole hypothalamus was ≈ 4 to 5 percent of capacity (*34*), indicating that only a small number of estrophilic cells were affected; and receptor occupation outside of the hypothalamus was below the limits of detection (*34*). These observations established that stimulation of the VMH alone is sufficient for activation of feminine sexual receptivity.

In parallel, it was also learned that the VMH is one of the sites for estrogen induction of progestin receptors (*7*); and implantation studies (*36*) have established that the VMH is by far the most sensitive site for progesterone to elicit its effects on sexual receptivity in estrogen-primed animals. Moreover, we have recently learned that a protein synthesis inhibitor, anisomycin, in the VMH blocks the activational effects of both systemically administered estradiol and progesterone (*37*). It thus appears that many of the essential chemical features of estradiol and progesterone action on feminine sexual behavior can be understood by elucidating the events evoked by these steroids within the VMH.

Mechanisms of Steroid Action on Nerve Cells

Steroid hormones can affect nerve cell activity (Fig. 5) by (i) direct action on membranes and (ii) an indirect action at the genomic level via the intracellular receptors localized by ^{3}H-labeled steroids (see above). In contrast to the intracellular receptors, the putative membrane receptors for steroids have not been clearly identified and their regional distribution is unknown.

Each time a neural steroid effect is identified, one important issue is to find out whether it occurs via the direct or indirect mechanism or whether it represents a primary or secondary effect of the hormone (*38, 39*). Direct effects are typically of short latency and brief duration. For example, 17β-estradiol hemisuccinate inhibits cell firing when applied iontophoretically in the preoptic area and hypothalamus (*40*). This effect is not produced by the 17α epimer (*40*), and in this respect the effect resembles the stereoselectivity of the intracellular estrogen receptors. Yet the millisecond latency and brief duration of these effects implies that another more direct mechanism is involved. In contrast, indirect effects are of longer latency and duration. Activation of sexual receptivity in female rats by estradiol, which has an 18- to 24-hour onset latency (*41, 42*) and which outlasts the removal of the estrogen stimulus by 24 to 36 hours (*42*), is blocked reversibly by inhibitors of RNA and protein synthesis (*2, 43*). Estradiol treatment has been shown to transiently stimulate RNA polymerase II activity in the rat hypothalamus (*44*).

There are also steroid effects that fall between these two extremes in terms of latency and duration. Such is the case for the effects of progesterone on sexual receptivity and proceptivity in estrogen-primed rats: onset latencies as brief as 30 minutes and a duration that outlasts by only a few hours the disappearance of progesterone from the circulation (*45*). Nevertheless, progesterone effects can be blocked reversibly by a protein synthesis inhibitor, anisomycin (*43*).

Another question pertaining to the mechanism of steroid effects is whether they are primary or secondary. Primary effects are those caused by an interaction of hormone with the responding cells. Secondary effects are those mediated by another hormone. For example, prolactin, which is secreted as a result of stimulation by estradiol, is implicated as mediator of a number of estrogen effects on neurotransmitter turnover in the brain (*46*). In a similar fashion, increased noradrenergic neural activity and release of noradrenalin from nerve endings appears to be responsible for increased accumulation of cyclic adenosine monophosphate (AMP) in estrogen-treated neural tissue (*47*).

Whether direct or indirect, primary or secondary, steroid effects on neural tissue must also be regarded in terms of their relation to neuronal electrical activity and synaptic transmission, which constitute the common currency by which the mechanisms of brain function and behavior are analyzed. Categories of demonstrated steroid effects (*38*) on cell functions related to neurosecretion and synaptic transmission include release of neurosecretory products (*48*), uptake (*49*), capacity for enzymatic inactivation (*50, 51*), biosynthetic capacity (*51, 52*), and receptor sensitivity to neurosecretion (*53, 54*). As indicated by the references, work on this topic has brought to light examples of each category for estrogens, progestins, androgens, and glucocorticoids. Therefore, we know in principle that these various aspects of neuronal function can be regulated by steroids. At the present time, however, knowledge of any steroid or steroid-sensitive brain region is insufficient to indicate which of the multiple effects of a given steroid are directly related to the steroidal activation of a behavior or neuroendocrine event.

Among the criteria for identifying such critical chemical events is the localization of the change within a neural pathway known to mediate the behavior. Two examples of recent progress in this area illustrate the problem. Androgen induction of cholinergic enzymes in the tracheosyringealis branch of the hypoglossal nucleus and the syrinx of the zebra finch is one example (*51*). These changes may not be a sufficient explanation for androgen activation of song, as female zebra finches do not sing in response to testosterone and yet do show the induction by testosterone of cholinergic enzymes in the syrinx (*51*). In this particular example, however, we know that the female brain differs morphologically from the male brain in that certain cell groupings within the song system are smaller in females than in males (*28*). Such morphological differences suggest that an insufficiency of cells or synaptic connections in the female brain might be at least a partial explanation for sex differences in singing ability independent of the activating action of the hormones (*55*).

Another example concerns the assessment of the role of changes induced by estradiol in the VMH for the activation of feminine sexual behavior in rats. As noted above, progestin receptor induction by estradiol is one function that appears to be associated with estrogen-sensitive neurons of the VMH, as well as other estrogen-sensitive cell groups of the hypothalamus and preoptic area. Induction of progestin receptors by estradiol is temporally correlated with the activation of sexual receptivity and proceptivity in female rats (*42*) and guinea pigs (*9*). Agents that block estrogen activation of sexual behavior, such as an

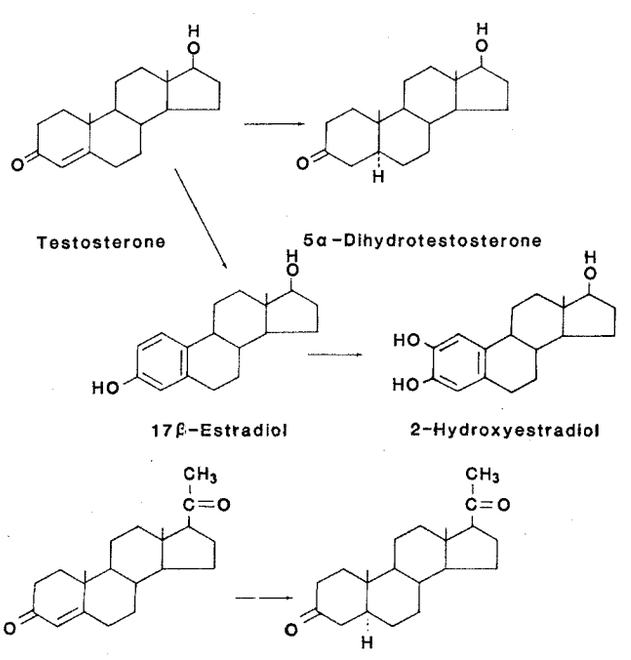

Fig. 4. Some of the major steroid transformations that occur in brain or pituitary tissue of the rat. [Reprinted from (*39*); courtesy of Raven Press]

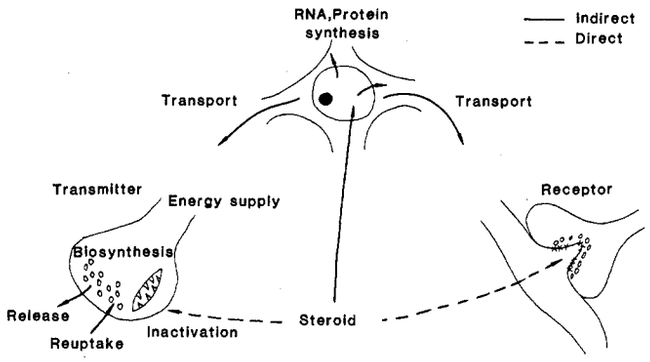

Fig. 5. Genomic and nongenomic effects of steroid hormones on pre- and postsynaptic events. Nongenomic effects (dashed line) may involve the action of the hormone on the pre- or postsynaptic membrane to alter permeability to neurotransmitters or their precursors or functioning of neurotransmitter receptors. Genomic action of the steroid (solid line) leads to altered synthesis of proteins, which after axonal or dendritic transport may participate in pre- or postsynaptic events. [Reprinted from (*39*); courtesy of Raven Press]

antiestrogen (*56*) and a protein synthesis inhibitor (*43*), interfere with progestin receptor induction as well. The correlation with sexual behavior is also seen in the realm of down regulation of progestin receptors (*57*). Large doses of progestin, which after 24 hours lead to refractoriness or reduced responsiveness to a second challenge of progestin, lead to a reduction in progestin receptor levels (*57*).

If progestin receptor induction were a necessary and sufficient condition for the activation of sexual behavior by estradiol, then understanding the mechanism of this process would focus on the mode of progesterone action. The cellular consequences of progesterone action are not known, but as stated above, it appears that progesterone facilitation of sexual behavior involves protein synthesis and that the proteins are synthesized rapidly and may have a rapid turnover (*43*). However attractive it may be to suppose a relatively simple connection between progestin receptor induction and sexual behavior, making such a connection appears somewhat premature. For one thing, in the absence of progesterone, high levels of estradiol priming activate one component of sexual behavior, namely, receptivity (*42*). There is no adequate explanation at the moment for the apparent override by estradiol of the requirement for receptivity. [There is, however, a stricter requirement for progesterone in the activation of the other component of sexual behavior, namely, proceptivity [see (*43*)]. Furthermore, estradiol has been shown to alter a number of cellular properties besides progestin receptors in the VMH. For example, estrogen treatment decreases glutamate decarboxylase activity (*58*) and increases muscarinic cholinergic receptor binding (*54*), as well as causing a decrease of type A monoamine oxidase activity (*59*). Thus, it is possible in this one critical estrogen-sensitive nucleus to see hormonal regulation of a neurotransmitter biosynthetic enzyme, a neurotransmitter degradative enzyme, and a class of postsynaptic neurotransmitter receptor sites; it is possible also that other chemical changes may be found. The distribution of the receptors within the VMH must be assessed as well as their relation to the projections from this nucleus to the mesencephalic central gray which has been shown to be an important supraspinal component of the circuit for the lordosis (receptive) response (*31*, *33*). Of particular interest and importance for further analysis is the report by Pfaff and Sakuma (*33*) that electrical stimulation of the ventromedial nucleus produces delayed (15 minutes to 1 hour) and prolonged (5 to 8 hours) facilitation of the lordosis response in estrogen-primed ovariectomized rats.

Although all the ramifications of androgen, estrogen, and progestin action in relevant brain nuclei are not yet clear, it is well to emphasize that our understanding of this aspect of the problem has come a long way—from the initial description of the distribution and properties of the neural gonadal steroid receptor systems to the stage where we can ask specific questions about the chemical plasticity of neurons in localized brain areas known to be important for the hormonal regulation of specified behaviors.

Sex Differences in Response to Gonadal Steroids in Rats

Besides yielding information on the mechanisms by which gonadal steroids alter neuronal processes and thereby affect behavior and neuroendocrine function in adult life, the rat has provided opportunities to study sex differences in response to hormones that are related to the occurrence of sexual differentiation.

The brains of adult male and female rats differ in their responses to gonadal steroids (*60*). Male rats are unable to show a luteinizing hormone surge after estrogen priming but are more responsive to testosterone priming of masculine sexual behavior. Gonadectomized male rats are less responsive than gonadectomized females to activation of receptivity by estradiol and are almost totally unresponsive to the synergistic effects of progesterone. These sex differences are determined largely through the influence of testicular androgen during late prenatal and early postnatal development (*60a*).

In terms of androgen and estrogen receptors and testosterone metabolizing enzymes, the brains of male and female rats are quite similar (Figs. 1 and 2). The induction of progestin receptors by estradiol and testosterone occurs in brains of both sexes, and at the level of the whole hypothalamus and preoptic area, no major sex differences in the estrogen-induced level of progestin receptors are known that would provide an explanation for the refractoriness of estrogen-primed males to progesterone (*61*).

In addition to these similarities of male and female brains, there are also a number of sex differences in hormone responsiveness. For example, the male preoptic area responds with increased electrical activity to testosterone application but not to estradiol (*62*). The male preoptic area of rats shows an androgen-induced increase in choline acetyltransferase activity, whereas the female preoptic area does not respond to the same androgen treatment (*63*). There are similar sex differences in the response of male and female mice to estradiol and testosterone with respect to increased type A monoamine oxidase activity (*64*).

The rather dramatic sex differences in hormone responsiveness can be accounted for in several ways. In the course of sexual differentiation, cells may have become selectively lost from male or female brains. Alternatively, cells may have become differentiated so as to respond differently to the hormone. The former possibility is consistent with observed morphological sex differences within the rat preoptic area which appear to involve differences in both cell number and cell size (*65*).

Ontogeny of Neural Gonadal Steroid Receptors and Hormone Effects

The rat has also afforded opportunities to study the relation between the ontogenesis of the gonadal steroid receptor systems, the occurrence of sexual differentiation, and the appearance of the characteristic effects of gonadal steroids in the mature brain (*60a*).

Neural estrogen, androgen, and progestin receptors in the rat are present in very low amounts just before birth, and they increase dramatically in the immediate postnatal period (*66-69*). Estrogen receptors are the first to increase, and this increase seems to be associated with the onset of sensitivity of the brain to the action of estradiol in triggering that aspect of brain sexual differentiation known as defeminization (*67*). The increase of neural androgen receptors occurs nearly a week later than the increase of neural estrogen receptors (*68*). The progestin receptor sites appear shortly after birth in the rat and increase in parallel with the postnatal increase of estrogen receptors (*69*). These receptors are not induced by estrogens until at least 8 to 10 days after birth (*69*). This inducibility is correlated in time with the first signs of the sexual reflexes (lordosis and ear wiggling) elicited by estrogen plus progesterone therapy (*70*). It seems likely that the emergence of progesterone receptor inducibility after the end of the critical period of sexual differentiation is a sign that the target cells are changing in their predominant mode of response to the hormone-receptor complexes from one involving growth and other structural changes to a mode in which the chemical features of target cells are reversibly modulated (*69*).

Neural Gonadal Steroid-Sensitive Cells Among Vertebrate Species

Having considered in some detail the distribution of gonadal steroid receptors and the cellular and chemical aspects of gonadal steroid actions on the brain in a few selected vertebrate species, I now summarize what is known for other species.

The mapping of estrogen- and androgen-sensitive cells in representative species of the major vertebrate classes by autoradiography has revealed a basic plan common to all. For the most extensively studied steroids—the estrogens—estrophilic neurons are found predominantly (*71-73*) in the medial preoptic area, in the tuberal hypothalamus, in specific limbic brain regions, such as the amygdala, and in a region of the mesencephalon deep to the tectum (Fig. 6). The regional neural distribution of neurons concentrating radioactivity injected as ^{3}H-labeled testosterone is similar, although not identical, to the pattern of estrophilic cells (*71, 72, 74*). The occurrence of aromatization in neural tissue makes interpretation of this distribution very difficult. More definitive studies of androgen-sensitive cells with ^{3}H-labeled 5α-dihydrotestosterone have been carried out thus far only in a reptile, *Anolis carolinensis* (*75*), and in the rat (*5, 6*). In these species, the distribution pattern of ^{3}H-labeled dihydrotestosterone neurons is similar, although not identical, to that produced by ^{3}H-labeled testosterone and estradiol.

With regard to the phylogenetic aspects of estrogen- and androgen-sensitive neurons and the ambiguity of interpreting the uptake of ^{3}H-labeled testosterone, we should consider the phylogenetic distribution of enzymes that metabolize it, namely the aromatizing enzyme system and the 5α-reductase. Both of these enzymatic activities have been detected in neural tissue of representative vertebrate species of all major vertebrate classes, from fish to humans (*11, 76*). The regional distribution of aromatase activity in brains of representative vertebrate species reveals an interesting, but so far unexplained, phylogenetic pattern. That is, whereas aromatase is consistently found in the limbic lobe and its counterparts, and in hypothalamus and preoptic area of virtually all species examined, there is aromatase activity in the mid-and hindbrain of fish and amphibia that is present in low levels or not detectable at all in reptiles, birds, and mammals (*76*).

Although androgen- and estrogen-sensitive neurons have a similar distribution in the brains of most vertebrates, there are a number of examples of divergences between distributions in certain species and some of these are already known to be functionally relevant. In the amphibian *Xenopus laevis*, only estrophilic neurons are found in torus semicircularis, ventral thalamus, ventral striatum, ventrolateral septum, and rostral amygdala, whereas only cells that concentrate ^{3}H-labeled testosterone (presumably androphilic) are found in the nuclei of the ninth and tenth cranial nerves and in the dorsal tegmental area of the medulla, regions that take some part in androgen-dependent mating vocalizations (*77*). In a reptile (*Anolis carolinensis*), more estrophilic than androphilic cells are found in the pallium, whereas androphilic cells are found in the absence of estrophilic cells in the mesencephalon (*74*). In the songbird *Poephila guttata* (zebra finch), androphilic cells exclusively are found in brain regions that are the site of the control of androgen-dependent song (*26, 55*).

Progestin receptors provide another emerging story regarding the interspecific similarities and differences in

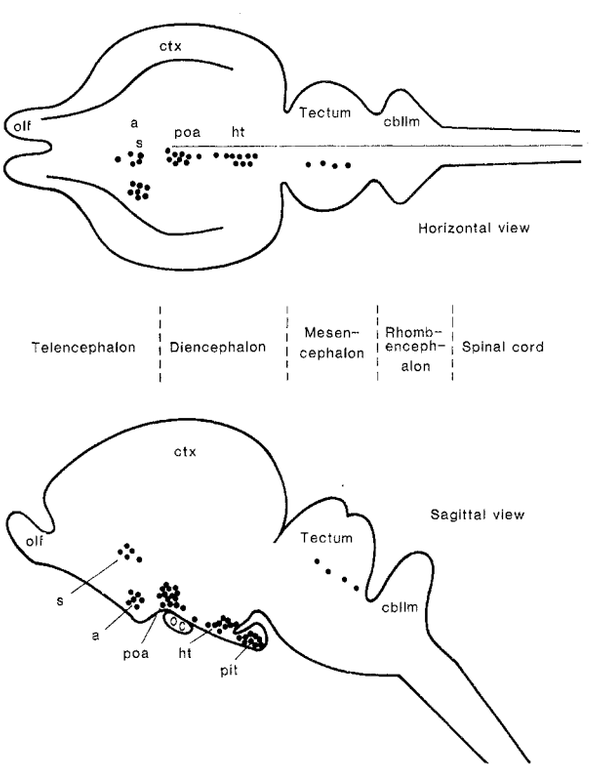

Fig. 6. Representation of the distribution of estrogen-sensitive neurons in the generalized vertebrate brain. [Courtesy of J. I. Morrell and D. W. Pfaff, Rockefeller University]

neural distribution of gonadal steroid responsive neurons. In the bonnet monkey (Fig. 3), progestin receptors are detected in the hypothalamus, where they are inducible by estradiol, and are absent in other brain regions; the preoptic area contains only a small number of progestin receptors, and these do not appear to be induced by estradiol (*78*). In the rat and guinea pig, estrogen-inducible progestin receptors are found in both hypothalamus and preoptic area and noninducible progestin receptors are also present in other brain regions such as amygdala and cerebral cortex (*8, 9*). Progestin receptors in the midbrain of the guinea pig are inducible by estradiol (*9*), whereas those in the midbrain of the rat (*78*) do not appear to be inducible (Fig. 3). In the reptile *Anolis carolinensis*, estrogen-inducible progestin receptors are found in the hypothalamic region, which includes the preoptic area as well as the hypothalamus; and noninducible receptors are found in a sample consisting of the rest of the brain (*10*). These differences are interesting in that estrogen-progesterone synergism is found in *Anolis* (*75*), as well as in rat (*42*) and guinea pig (*57*) where it facilitates feminine sexual behavior, whereas progesterone does not facilitate feminine sexual behavior in estrogen-primed monkeys (*79*). It is not clear whether the most relevant aspects of these differences in distribution relate to the presence or absence of the noninducible progestin receptors or to as yet unknown differences in the distribution of estrogen-inducible progestin receptors within the hypothalamus.

With respect to sex differences, the neural distribution of gonadal steroid-sensitive neurons is characteristic of the species, but, for the most part, independent of the genetic or phenotypic sex (*71, 72*). For example, the distribution of testosterone- and estradiol-sensitive neurons does not differ between the sexes in the rat (Figs. 1 and 2) or in the amphibian *Xenopus laevis* (*77*). And as noted above, the same appears to be true of the capacity of the rat brain to convert testosterone to dihydrotestosterone or to estradiol (Figs. 1 and 2). Yet there are examples of quantitative sex differences in steroid receptor levels. In the zebra finch, more androgen-concentrating neurons are found in males than in females in two areas of song control: the hyperstriatum ventrale pars caudale and the magnocellular nucleus of the anterior neostriatum (*28*). In the rat spinal cord, males contain a group of androgen-concentrating neurons in the lumbar region which innervate striated muscles of the penis and which do not appear to be present in females (*55*). It would not be surprising if more such examples were to be discovered.

Comparative Aspects of Gonadal Steroid Action on the Brain

The specific neural circuits related to reproductive behavior and their steroid sensitivity are expressions of the genetic repertoire of each species. Although a basic plan of gonadal steroid-sensitive neural systems is recognizable across vertebrate classes (see above), there are also examples of species-specific patterns of steroid-sensitive neurons which are superimposed on the basic plan. The species-specific nature of certain aspects of steroid receptor distribution may be one factor that accounts for the rather remarkable diversity present, even among mammals, with respect to (i) the kinds of hormones that activate sexual behavior or cause sexual differentiation and (ii) the extent to which particular behaviors undergo sexual differentiation. Illustrations of this diversity and ways of accounting for it at the cellular level are discussed below.

The first category of diversity is that mammalian species and even strains within species differ in the degree to which the adult males or females display bisexuality in their sexual behavior. According to Goy and Goldfoot (*80*), this appears to be the result of the fact that the extent of hormonal masculinization and defeminization during development differs among species. Masculinization is defined as the enhancement during development of masculine traits; defeminization is the suppression in development of feminine traits, especially those that are normally activated in adult life by estrogens and progestins. In rats, females undergo some degree of masculinization and are more bisexual than males, whereas males are extensively defeminized. In rhesus monkeys, males do not undergo defeminization, but are masculinized and become more bisexual than females.

A second category of diversity is that mammals also differ in the kinds of hormones that activate sexual responses. Among females, the major interspecific difference is whether progesterone synergizes with estradiol in affecting sexual behavior. Baum (*81*) has pointed out that in the ferret, the rhesus monkey, and the prairie vole (*82*), in contrast to rodents, sheep, and dog, progesterone does not act synergistically with estradiol to increase sexual receptivity. In fact, progesterone in these species may even decrease the attractiveness of the female to the male (*81*). Among males, the major interspecies difference is in the dependency of copulatory behavior on androgen receptors, or on estrogen receptors and aromatization (*60*). Androgen receptor mechanisms appear to predominate in the response of the rhesus monkey and guinea pig to testosterone, whereas aromatization and estrogen receptors predominate in the activation of masculine sexual behavior in the rat, hamster, and red deer stag (*60*).

The third category of diversity is that mammals differ in the kinds of hormones that promote sexual differentiation (*60, 60a, 81*). Defeminization in rats is mediated by aromatization and estrogen receptors. A similar situation applies for masculinization and defeminization in hamsters. In guinea pigs, masculinization is produced via the androgen receptor pathway, whereas defeminization probably occurs via the aromatization pathway. In rhesus monkeys, where defeminization does not appear to occur, masculinization occurs via the androgen pathway.

As stated above, these variations among species may be understood as expressions of the genetic program of each species. In accounting for the diversity noted above, we might suppose that in each species neurons of different hormonal sensitivities are programmed to develop within the appropriate brain circuits; they may be further programmed to participate first in the development (that is, sexual differentiation) of these circuits and later in their activation during adult life. Related to this notion is a key generalization that activator hormones are also organizer hormones (*83*). This generalization appears to hold quite well for masculinization; for example, in hamster, guinea pig, and rhesus monkey, the masculinization and activation of masculine behavior both involve the same pathway. In the case of the hamster, it is aromatization and estrogen receptors; for the guinea pig and rhesus monkey, the pathway is that involving androgen receptors (*60, 81*).

In the case of defeminization, the situation is more complex, although not necessarily different. This is because defeminization is the suppression in development of responses normally activated by estrogens in adulthood in normal females. There are, however, indications for a limited number of species that defeminization is mediated by aromatization [such as in the rat, hamster, and guinea pig; see (*60, 60a, 81*)]. If this were to hold as a generalization, then it would be true that both defeminization and ac-

tivation of feminine sexual behavior involve estrogens.

Another aspect of defeminization, which makes it more complex than masculinization, is that it does not appear to occur universally among mammals (*60, 81*). The simplest explanation for this is to suppose that in those species lacking defeminization the developing neural circuits are insensitive to estrogens or lack aromatizing enzymes during the critical period, even though they may subsequently become sensitive to estrogens for the activation of feminine sexual behavior. This hypothesis is testable once we have access to the neural circuits involved in feminine sexual behavior in species such as the ferret and rhesus monkey in which defeminization is lacking. Baum (*81*) has pointed out an apparent correlate of the absence of defeminization which is well worth bearing in mind, namely, that in these two species where defeminization is known to occur, it is also known that progesterone does not show synergistic effects with estradiol on feminine sexual behavior. The key to this puzzle and to the nature of defeminization may therefore be tied up in some way with progestin-sensitive neurons.

The sequence of events proposed to explain lack of defeminization would also provide a basis for understanding the type 2 category of sexually dimorphic behaviors referred to earlier. The type 2 behaviors are those that become sexually dimorphic independently of steroids, yet which are activated by gonadal steroids in adult animals.

In a like manner, type 3 behaviors, which are hormone-sensitive during sexual differentiation and yet independent of hormones in adult life, might reflect the presence of hormone-sensitive cells in development which lose hormone sensitivity in adult life. The disappearance of estrogen receptors from the rat cerebral cortex during the third postnatal week of life (*84*) indicates that such loss of hormone sensitivity does occur.

Conclusions

In conclusion I suggest a number of generalizations that may be applicable to understanding neural actions of gonadal steroids across vertebrate classes and among mammals.

1) Neurons sensitive to gonadal steroids occupy prominent positions within neural circuits which mediate stereotyped components of behaviors broadly related to the reproductive process; these include mate calling and response to calls in frogs, vocalization in songbirds, and lordosis behavior in rats. Steroid-sensitive neurons have been found in these and other cases to occupy positions of motoneurons (*55, 85*), as well as neurons of sensory projection areas (*85*), but also to lie in a position outside of the actual reflex arc (*33*) from which a facilitatory or inhibitory influence is exerted. As we have seen from the discussion of cellular mechanisms above, various means are available by which steroids can enhance synaptic function. They can increase neurotransmitter biosynthesis, decrease uptake or degradation, or enhance receptor sensitivity to neurotransmitters. Also to be kept in mind is the possibility of disinhibition through negative steroid effects on neuronal excitability and neurotransmission in inhibitory neurons. In these instances, synaptic efficacy of inhibitory neurons might be decreased by steroid influences that would increase transmitter biosynthesis, increase transmitter reuptake or degradation, or decrease receptor sensitivity to some neurotransmitters. Current knowledge concerning steroid effects consists in the demonstration that steroid treatment causes changes in synaptic properties and function even though such changes cannot be used at present to explain the steroidal activation of a neural circuit.

2) Sensitivity to gonadal steroids may reside within groups of developing neurons that are destined to form the circuitry for behaviors related to the reproductive process or to other functions as well, such as performance of rats in mazes (*86*). As indicated by MacLusky and Naftolin (*60a*) the actions of gonadal steroids to promote sexual differentiation involves cellular events, such as growth facilitation, which are more characteristic of developing neurons than of adult neurons. There is a transition period in the maturation of these hormone-sensitive neurons in which the effects of gonadal steroids related to sexual differentiation wane and the reversible activational effects of these steroids emerge, reflecting the continuing differentiation of the responsive neurons.

Various programs may be operating in individual species for the expression or nonexpression of hormone sensitivity at various stages of development. These programs involve the types of hormonal sensitivity (androgen, estrogen, progestin) that are expressed and the developmental stage at which such expression occurs. I have suggested that variability of expression may account for the rich diversity of hormone sensitivity evident among mammalian species with respect to the activation and sexual differentiation of masculine sexual behavior, the occurrence or nonoccurrence of defeminization and of the neural estrogen-progestin synergism on feminine sexual behavior, as well as the existence of type 1, type 2, and type 3 classes of sexually dimorphic behavior.

In the light of these generalizations, we can consider our own species. The human, like the rhesus monkey, is a species in which masculinization, rather than defeminization, appears to be the predominant mode of sexual differentiation (*60*). It seems reasonable that the neural substrate for gonadal steroid responsiveness is represented in the human brain in much the same way that we know it to be represented in the brains of the rhesus monkey and bonnet monkey—with a basic plan like that of other vertebrates but with unique features of hormone response, such as the distribution of neural progestin receptors (*78*). Other articles in this issue (*87*) elaborate on the extent to which we are able to recognize, in spite of the environmental influences of learning, the components of human behavior which are influenced by hormones during development and in adulthood.

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88. I thank Dr. G. P. Smith of Cornell University Medical College and Drs. J. I. Morrell and D. W. Pfaff of Rockefeller University for critical reading of the manuscript. This work was supported by grant NS07080 from the National Institutes of Health and by grant RF70095 from the Rockefeller Foundation.