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Gonadal Hormones and Brain Development: Cellular Aspects of Sexual Differentiation

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SYNOPSIS. Sexual differentiation of the neural control of reproductive function with respect to both gonadotropin secretion and sexual behavior is thought to result from exposure of the brain to testicular androgens during a very restricted or critical period of CNS differentiation and development, when the tissue is competent to respond to the hormone, and after which it is refractory or responds in a reversible manner. This paper reviews the cellular aspects of sexual differentiation with particular emphasis on the morphological expression of the gonadal hormonal effects in the adult brain. It presents experimental evidence for the morphogenetic basis for the observed steroid effects by showing how the addition of steroid to undifferentiated hypothalamic cultures produces a selective neuritic response that is steroid-dependent. These results suggest that since afferent axonal input and temporal factors are critical for dendritic and synaptic differentiation, steroid-induced variations in neuritic development could result in gender-specific responses seen in sexual differentiation of reproductive function.

INTRODUCTION

Sexual differentiation of the neural control of reproductive function in mammals with respect to both gonadotropin secretion and sexual behavior is thought to result from exposure of the brain to testicular androgens during a very restricted or critical period of Central Nervous System (CNS) differentiation and development, when the tissue is competent to respond to the hormone, and after which it is refractory or responds in a reversible manner. Although most of the studies on sexual differentiation of the brain have been carried out in rodents, the basic concepts have been shown to exist in many other species, including primates. It must always be kept in mind, however, that although the underlying principles of hormonal action are probably valid for all of them, the physiological functions involved, the neural sites affected, the timing of the critical period and even the very hormone(s) responsible may vary widely among species.

For the rodent in whom the influence of gonadal hormones on sexual differentiation of the brain has been most extensively studied (see Gorski, 1971, 1973, for review), the critical period for steroid sensitivity occurs perinatally, extending into the early neonatal period. In the rat, sensitivity of the brain to steroid may be shown to extend from the 18th day of gestation to the 11th postnatal day (Barraclough, 1971; Lobl and Gorski, 1974). Single subcutaneous steroid injections or neonatal castration, however, are maximally effective only during the first five postnatal days—the period which may thus be considered as the physiological one.

During the critical period, androgens masculinize the neonatal male rodent with respect to the post-pubertal development of masculine behavior and the non-cyclic or tonic patterns of gonadotropin release. It is generally presumed that in the neonatal female, the absence of testicular secretions results in the development of the female pattern of sexual behavior and the

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cyclical pattern of gonadotropin release necessary for ovulation. The permanent post-pubertal alterations in reproductive function induced by neonatal androgenization of the rodent brain has led to the concept that, regardless of genetic sex, the newborn brain, though sexually undifferentiated and bipotential, is intrinsically organized to support the cyclical or female pattern of reproductive activity. Thus androgenization of the neonatal female and castration of the neonatal male have been shown to profoundly disturb the development of those regulatory mechanisms crucial for the gender-specific responses. Neonatally androgenized females are rendered anovulatory and sterile; the pattern of gonadotropin release is masculinized (tonic) and there is both an absence of female behavior and an increased display of male behavior (Barraclough, 1961; Barraclough, 1967). Males castrated neonatally during the critical period will support ovulation of ovarian grafts, exhibit a cyclical (female) release of gonadotropins and a female pattern of sexual receptivity in the presence of a normal male.

POSSIBLE MODES OF STEROID ACTION

How androgen (testosterone) elicits its irreversible effect is poorly understood, although increasing evidence suggests that the initial mode of testosterone action may involve its intraneuronal conversion (aromatization) to estrogen (estradiol) in such neural sites as the preoptic area, hypothalamus and the amygdala (Naftolin *et al.*, 1971; Weisz and Gibbs, 1974; Reddy *et al.*, 1974; Lieberburg and McEwen, 1975; Lieberburg *et al.*, 1977). That the androgen (testosterone) effect may be mediated by conversion to estradiol is supported by studies showing that the administration of estradiol during the critical period mimics the effects of androgen: blocking ovulation in the genetic female and masculinizing the patters of neural differentiation in the neonatally castrated male (Barraclough and Gorski, 1961; Barraclough, 1967). Neonatal treatment of female rats, furthermore, with a non-aromatizable androgen such as dihydrotestosterone (DHT) has been ineffective (Luttge and Whalen, 1970; McDonald *et al.*, 1970; McDonald and Doughty, 1974) or only partially effective Gerall *et al.*, 1975) in altering the normal female pattern of neural differentiation. Similarly, antagonism of both testosterone-induced (McDonald and Doughty, 1973; Doughty and McDonald, 1974; McEwen *et al.*, 1978; Vreeburg *et al.*, 1977) and estrogen-induced (Doughty *et al.*, 1975) masculinization of the brain by anti-estrogens, which compete with estradiol for the receptor sites to block its physiological action, and the attenuation of the masculinizing effects of both endogenous and exogenous testosterone by aromatizing enzyme inhibitors (Booth, 1977; McEwen *et al.*, 1978; Vreeburg *et al.*, 1977), which block the conversion of testosterone to estradiol, further supports the hypothesis that sexual differentiation in rodents depends on the production of small amounts of estradiol from testosterone.

THE DEMONSTRATION OF STEROID RECEPTOR SITES AND THEIR ROLE

That these steroids may influence the neural substrate has been shown by the demonstration of selective neuronal nuclear uptake and retention of the gonadal hormones. Target cells concentrating estradiol and testosterone with the same distribution as in the adult have been demonstrated by ^{3}H-steroid autoradiography in the hypothalamus, preoptic area and the amygdala of the two-day-old neonatal rat (Sheridan *et al.*, 1974*a,b*) and estrogen-concentrating neurons have been found in the cerebral cortex of neonatal rats (Gerlach, Plapinger and McEwen, personal communication). The distributional pattern of the steroid concentrating neurons in the brain, furthermore, is qualitatively the same in male and female rats. High affinity, distinct androgen- and estrogen-binding macromolecules, putative "receptors" with properties indistinguishable from those of adult animals have been shown in cytosol and nuclear extracts of the same regions of the neonatal rat and mouse (Barley *et al.*, 1974; Fox, 1975 *a,b,*;

McEwen *et al.,* 1975; Attardi and Ohno, 1976; Westley and Salaman, 1977). In tissues of adult vertebrates estrogen is believed to act through binding to specific cytoplasmic receptor sites, which subsequently translocate to the cell nucleus and initiate changes in nucleic acid and protein systhesis (Gorski and Gannon, 1976). In the rat uterus, the most extensively studied mammalian estrogen-sensitive tissue, the sequence of events mediating the response is highly dependent on early protein synthesis (Beaulieu *et al.,* 1972; Gorski and Morgan, 1967; Notides and Gorski, 1966), a small number of labile cytoplasmic and non-histone proteins, possibly regulatory in function, being synthesized rapidly following exposure to the hormone (Notides and Gorski, 1966; Beaulieu *et al.,* 1972; King *et al.,* 1974; Cohen and Hamilton, 1975*a,b*). The changing pattern of steroid uptake during the first month of life has suggested maturation of the receptor (Kulin and Reiter, 1972; Plapinger and McEwen, 1973; Kato *et al.,* 1974). Since steroid effects are believed to be mediated through steroid receptors, it has been suggested that androgenization may result from interference with maturational or metabolic aspects of the steroid receptor system (Flerkó *et al.,* 1969; Vértes and King, 1971; Gorski, 1971).

THE POSSIBLE IMPORTANCE OF ESTROGEN PER SE

While it is generally accepted that androgen is necessary for sexual differentiation of the brain, nothing is known about steroid requirements, if any, for the normal development of the female brain. The prevailing opinion generally holds that androgen alone is the determining factor in sexual differentiation, feminization merely representing the (passive) emergence of the anhormonal state; the intrinsic pattern of neural organization common to the brain of both genetic sexes. It is generally assumed that the neonatal female brain is protected from masculinization by the high levels of maternal estrogen circulating during the perinatal period, through functional inactivation by binding to the fetoneonatal extracellular-binding-protein, alpha-fetoprotein (McEwen *et al.,* 1975). Testosterone, on the other hand, which is not bound by alpha-fetoprotein, has free access to the brain where it is presumably aromatized to estradiol in those regions of the brain containing aromatizing enzymes, regions implicated in the neuroendocrinology of reproductive activity and sexual behavior. The true extent to which estradiol is functionally inactivated by alpha-fetoprotein, however, is unknown.

Speculation that estrogen may have a more fundamental and general role in CNS development, however, is increasing, based on the demonstration of estradiol receptors in the cerebral cortex of the neonatal rat (Barley *et al.,* 1974; McEwen *et al.,* 1975). Unlike the adult in whom estradiol receptors are most concentrated in the hypothalamus and the amygdala and virtually absent from the cerebral cortex (Eisenfeld, 1970; Zigmond and McEwen, 1970; Pfaff and Keiner, 1973), neonatal rats and mice of both sexes contain equal amounts of high affinity estradiol receptors in both hypothalamus and cerebral cortex in concentrations similar to that of the adult female hypothalamus (MacLusky *et al.,* 1976). The significance of the cortical receptors is unknown. Since the absence of aromatizing enzymes in the cortex (Liberburg and McEwen, 1975) precludes a testosterone-mediated estrogen effect, suggesting that the cortex is unlikely to be involved in the process of masculinization, Barley *et al.* (1974) have raised the possibility that estradiol itself may be involved in an unspecified "organizing" effect on the brain. It is not at all clear, however, whether these estradiol receptors are at all functional, since alpha-fetoprotein is present in both males and females during the neonatal period.

Tangential evidence, however, supports the hypothesis that the estradiol receptors in the neonatal brain may mediate developmental effects of estrogen, as well as those of testosterone. While it may well turn out to be purely fortuitous and circumstantial, the ontogenetic patterns of various morphological, biochemical and

physiological aspects of the neonatal period, viewed in relation to one another, would argue for an active morphogenetic role for estrogen in the CNS. Unlike the binding by the hypothalamic estradiol receptors which are maintained throughout adulthood, neonatal estradiol binding by the cerebral cortex has been shown to reach its maximum around day 10 (MacLusky *et al.*, 1976), the cortical ^{3}H-bindir g sites declining rapidly thereafter between days 10 and 21 to virtually undetectable levels (McEwen *et al.*, 1975). Of great significance and interest in this regard is the fact that the cerebral cortex of the rodent, like the hypothalamus/preoptic area, is relatively undifferentiated at birth. Similarly, it exhibits a critical period of postnatal development around the tenth day (Flexner, 1952), which heralds the onset of rapid differentiation and the intense sprouting of neurites. The cortex is unmyelinated on day 10, and critical aspects of morphological, biochemical and functional differentiation develop during the second and third week of postnatal life (Bass *et al.*, 1969). In this CNS region, therefore, as in the hypothalamus/preoptic area, the presence of estradiol receptors appear to coincide with the critical period for cerebral differentiation. Of particular note, furthermore, is the fact that unlike the hypothalamus/preoptic area in which steroid sensitivity is a characteristic of the adult, estradiol receptors appear to be present in the cerebral cortex *only* during the critical period.

During this same postnatal period, moreover, alpha-fetoprotein decreases linearly from its very high concentration at birth (half-life of 4 days) to levels of zero at weaning and thereafter (Vannier and Raynaud, 1975). Serum estradiol levels, on the other hand, in *both* male and female neonatal rats, after being elevated for the first 2 postnatal days (maternal) and then falling rapidly, increase abruptly between day 9 and 19 (with a peak around day 10) to levels which are never subsequently observed during life (Döhler and Wuttke, 1975). Declining levels of the estradiol-binding alpha-fetoprotein thus occur in the presence of very high serum levels of estradiol; levels which peak around days 10 and 11 and which are found only during the second and third postnatal week. This raises serious speculation that the increasing levels of *unbound* estradiol might then be free to exert a morphogenetic effect in regions of the CNS shown to contain estradiol receptors during their critical period, *viz.*, hypothalamus and cerebral cortex.

These speculations find support in the observations of estrogen-induced enhancement of cortical maturation (Heim and Timiras, 1963; Heim, 1966) and of myelinogenesis (Curry and Heim, 1966) and of the permanent inhibitory action of neonatal estrogen on the incorporation of ^{3}H-lysine into the pyramidal cells of the cerebral cortex (Litteria and Thorner, 1976; Litteria, 1977). Ohno *et al.* (1975), moreover, have proposed that neonatal imprinting by steroid might be a prerequisite for both masculinization and feminization of the brain, by demonstrating the total absence of sexual behavior in the androgen-insensitive male mouse with the Tfm (testicular feminizing) mutation. Diamond *et al.* (1971) have suggested, moreover, that the sex steroids may protect the cerebral cortex from the developmental retardation generally associated with an impoverished environmental condition, as determined by measurements of cortical thickness. That estrogen may have a general role in axonal growth patterns in regions containing estradiol receptors is also suggested by Nance *et al.* (1975) who propose that the observed estrogen sensitization of septal-lesioned male rats might be related to estrogen-induced recovery by such a mechanism as axonal sprouting.

MORPHOLOGICAL ASPECTS OF SEXUAL DIFFERENTIATION

Although the developmental importance of the gonadal steroids on sexual differentiation of the brain is well-documented, the neural site(s), substrate and morphogenetic aspects of these effects are largely unknown. Numerous studies (Gorski, 1966; 1970; 1971; Raisman and Field, 1971*a*; Everett, 1972) have impli-

cated the hypothalamus and such extrahypothalamic CNS regions as the preoptic area and the amygdala in the neural regulation of LH release and sexual behavior, regions which in the rodent are all poorly differentiated at birth. Nadler (1968, 1972, 1973), Hayashi and Gorski, (1974) and Lobl and Gorski (1974) have shown that testosterone implants in the preoptic area and the hypothalamus of the neonatal female cause masculinization but their results also suggest that the effect may not be limited to the hypothalamus alone. During the development of the preoptic area, furthermore, a key region for the cyclical release of LH, ovulation and sexual behavior (see Gorski, 1974, for review), Reier *et al.* (1977) have shown a close correlation between structural maturation of its neurons and the surrounding neuropil and the period of androgen sensitivity, the major phase of cytoplasmic differentiation occurring *after* the critical period, between the fifth and tenth postnatal days. Thus the absolute of physiological critical period in this CNS region would appear to correspond morphogenetically with the end of the neuroblast or undifferentiated phase. This is an obviously vulnerable period, characterized by the onset of the intense metabolic and biosynthetic activities which precede morphological differentiation and which might well be sensitive to the steroid effects. These observations suggest that in the preoptic area, at least, the onset of both structural and functional maturation coincides with the end phase of the critical period.

SEXUAL DIMORPHISM OF STRUCTURE AND FUNCTION

That the gonadal hormones may induce a permanent structural alteration of the neural substrate for sexual differentiation has been supported by observations of various types of steroid-dependent morphological differences or *sexual dimorphism* in various regions of the adult CNS implicated in reproductive function (Table 1). The morphological consequences of experimental perinatal exposure to steroid have included differences in: (1) individual neuronal nuclear and nucleolar size in the preoptic area, ventromedial nucleus and the amygdala (Ifft, 1964; Pfaff, 1966; Dörner and Staudt, 1968, 1969; Hellman *et al.*, 1976; Staudt and Dörner, 1976); (2) ultrastructural features of neuronal organelles in the arcuate nucleus (King, 1972) and in its types of synaptic terminals and synaptic vesicles (Ratner and Adamo, 1971; King, 1972; Matsumoto and Arai, 1976); (3) the patterns of synaptic organi-

TABLE 1. *Androgen-dependent sexual dimorphism in the CNS.*

Structure	Region	Animal	Authors
Neuronal nuclear and nucleolar size	Preoptic area	Rat	Ifft, 1964; Pfaff, 1966
	Ventromedial		Dörner and Staudt, 1968, 1969
	Amygdala		Hellman *et al.*, 1976 Staudt and Dörner, 1976
Neuronal organelles (ultrastructural)	Arcuate	Rat	King, 1972
Synaptic vesicles	Arcuate	Rat	Ratner and Adamo, 1971
Synaptic terminals			King, 1972 Matsumoto and Arai, 1976
Synaptic organization	Preoptic area (non-amygdaloid afferents)	Rat	Raisman and Field, 1971*b*, 1973
Dendritic branching patterns	Preoptic area Suprachiasmatic	Hamster, Rat	Greenough *et al.*, 1977
Nuclear volume (gross)	Nucleus robustus archistriatalis (RA)	Songbird	Nottebohm and Arnold, 1976
	Hyperstriatum ventrale, pars caudale (HVc)		
	Preoptic area	Rat	Gorski *et al.*, 1977, 1978

zation (ratio of synapses ending on the shafts *vs.* spines of dendrites) of the non-amygdaloid (non-*stria-terminalis*) afferents in the dorsal preoptic area (Raisman and Field, 1971*b*, 1973); (4) dendritic branching patterns in the medial preoptic area (Greenough *et al.*, 1977) and in the suprachiasmatic nucleus (Greenough and Carter, personal communication); and (5) differences in the gross volume of the medial preoptic area (Gorski *et al.*, 1977*a*; Gorski *et al.*, 1978). This last example, a finding so gross that the gender of the adult rat may be determined by observation of thionin-stained 60 mμ sections of the medial preoptic area with the naked eye, resembles the earlier described striking increase in gross nuclear volume of two vocal control centers (HVc and RA) in male songbirds (Nottebohm and Arnold, 1976) which participate in singing, a learned but clearly androgen-dependent behavior.

Various studies have also demonstrated biochemical and pharmacological sex differences in the rat hypothalamus as a consequence of normal sexual differentiation or in response to androgenization of the female or castration of the male during the critical period (Hardin, 1973*a* and *b*; Ladosky and Gaziri, 1970; Litteria, 1973; Moguilevsky *et al.*, 1971).

The precise sexual dimorphic function, if any, subserved by all these observed structural differences in the brain is unknown. As if to emphasize this, no qualitative or quantitative ultrastructural differences were observed during the normal development of the male and female preoptic area (Reier *et al.*, 1977).

These increasingly numerous examples of androgen-dependent structural dimorphism in regions of the adult brain implicated in reproductive function, have led to the hypothesis that differences in neural connectivity (circuitry) may form the neural substrate for sexual differentiation. Yet, despite such demonstrations of neural plasticity and reorganization of neuropil during the critical period, these sexually dimorphic structures merely represent the final results of the steroid effects. They tell nothing about the morphogenetic mechanisms which produced them.

MORPHOGENETIC EFFECTS OF ESTRADIOL AND TESTOSTERONE *IN VITRO*

In studies designed to elucidate the cellular events involved in the neurogenesis of sexual differentiation, I have been studying the influence of exogenous steroids on the development of the hypothalamus and preoptic area in a tissue culture system (Toran-Allerand, 1976). Estradiol-17β and testosterone, added exogenously to explants of the newborn mouse hypothalamus preoptic area of both genetic sexes, produce a selective acceleration and enhancement of the proliferation of neuronal processes or neurites, which are restricted to specific regional levels and to specific areas within those levels. The steroid response, which is most striking in the preoptic area and infundibular/premamillary regions, is characterized by extensive neuritic proliferation and the formation of dense plexuses of neuronal processes which extend far into the distal outgrowth (Figs. 1 and 2). Relatively few neurites appear to contribute to plexus formation, suggesting that the phenomenon may involve the induction of branching in the neurites of small numbers of steroid-sensitive neurons rather than the generalized response of all neurons. In support of this hypothesis was the subsequent demonstration by ^{3}H-estradiol autoradiography (Gerlach, Toran-Allerand and McEwen, unpublished) of a subpopulation of steroid concentrating neurons which, as *in vivo,* are discretely localized in cultures of the preoptic area and infundibular/premamillary levels but not at the level of the anterior nucleus of the hypothalamus—a non-steroid responsive region *in vitro.*

Conversely, reduction of the steroid endogenous to the horse serum component of the nutrient medium by antibodies specific to estradiol-17β (Toran-Allerand, 1976), or by charcoal/Dextran or Sephadex LH-20 extraction (McEwen and Toran-Allerand, unpublished) produced the exact opposite of the steroid response.

This effect was characterized by a reduction and retardation of neuritic outgrowth only in regions previously shown to be steroid-sensitive. Such studies provide additional support for the hypothesis that estrogen *per se* may have a role in neural differentiation other than, and in addition to, that restricted to sexual differentiation.

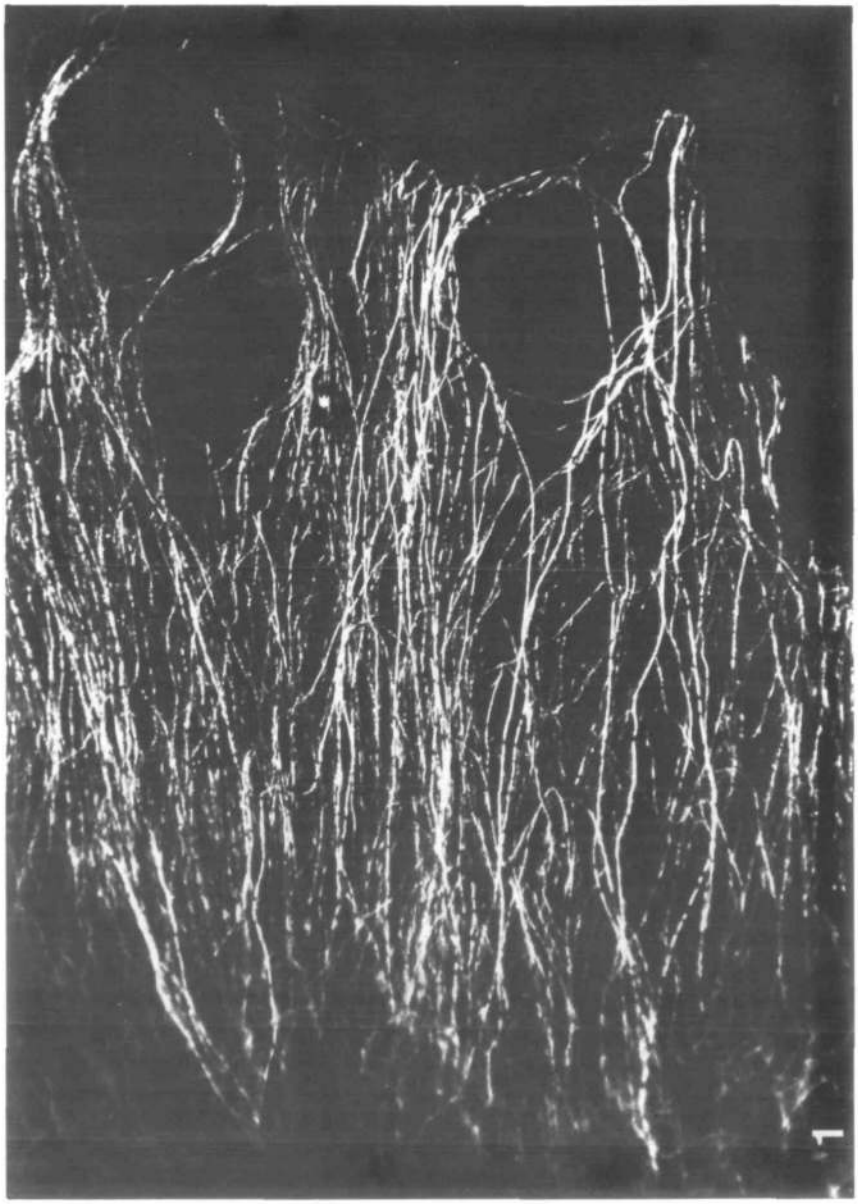
The addition of testosterone alone, in the absence of endogenous estradiol, produced no significant enhancement of the normal neuritic response while DHT, a non-aromatizable androgen, was without observable effect even in the presence of endogenous estradiol (Toran-Allerand, in preparation). These results are consistent with the hypothesis that testosterone exerts its effect in the brain by its aromatization to estradiol-17β. In further support of a specific estrogen effect and of the possible involvement of the intranuclear estradiol receptors demonstrated by ^{3}H-autoradiography are the observations (Toran-Allerand, unpublished) that the non-steroidal anti-estrogen, CI 628, inhibits by at least 50% the neuritic outgrowth induced by the estrogens endogenous to the horse serum. These findings are in keeping with the observations that anti-estrogens attenuate the masculinizing effects produced by testosterone (McDonald and Doughty, 1973; Doughty and McDonald, 1974; McEwen *et al.*, 1977; Vreeburg *et al.*, 1977).

THEORETICAL CONSIDERATIONS: IMPLICATIONS FOR THE NEUROGENESIS OF SEXUAL DIFFERENTIATION

These experimental observations may be viewed in terms of some general principles of CNS differentiation in order to provide a hypothesis for the morphogenetic basis of sexual differentiation. Differentiation and development of the CNS is based on well-ordered sequences of interlocking phenomena where timing is critical and each aspect is seemingly dependent on the entire sequence of events that have preceded it. Alterations in the timing of sequential phenomena can thus have profound physiological effects without significantly altering the basic morphology which can thus appear "normal." Morphological differences *may* exist, however, but be expressed in more subtle ways such as in the patterns of dendritic differentiation and synaptic organization which need not be abnormal in themselves, yet result in profound functional differences.

Studies on the ontogeny of dendritic differentiation throughout the CNS (Morest, 1969; Kornguth and Scott, 1972) have led to the concept that patterns of dendritic differentiation and synaptic organization are induced by their afferent axonal input in addition to the cell's genome. Morest (1969) has shown that dendritic differentiation generally follows that of the axon and a close temporal relationship exists between dendritic differentiation and the appearance of afferent endings in their immediate vicinity. Gottlieb and Cowan (1972) have suggested in studies on the development of the dentate gyrus that since the amount of post-synaptic space available appears to be strictly limited and relatively constant for each neuronal type, the repartition of synaptic sites between different groups of converging axons is determined competitively on a temporal basis. The spatial distribution of synapses, therefore, appears to reflect the differential growth rates of specified axons and the relative number of each axonal category present during synaptogenesis. The observations of Raisman and Field (1971*b*, 1973) and Greenough *et al.* (1977) on androgen-dependent, sexually dimorphic aspect of synaptic topography and dendritic differentiation become further important because patterns of dendritic branching, dendritic spine density and synaptic organization have been shown throughout the CNS to exhibit considerable postnatal plasticity. Environmental factors such as visual deprivation (Globus and Scheibel, 1967; Lund and Lund, 1972) and endocrine factors, such as hypothyroidism (Rebière and Legrand, 1972), acting postnatally can modify them.

The timing or rate of axonal development shown to be critical for the establishment of neuronal interactions *in situ* appears to be influenced *in vitro* by the gonadal



FIGS. 1 and 2. Photomicrographs of a silver-impregnated, homologous (mirror) explant pair of cultures of the newborn mouse hypothalamus, 19 days *in vitro* (infundibular/premamillary level). Holmes reduced silver nitrate method, Darkfield, × 125. FIG. 1. Control exposed only to steroid endogenous to the serum. Numerous silver-impregnated axons course outward from the margin of the explant. FIG. 2. Estradiol-17β 100 ng/ml. The neuritic outgrowth of the homologue exhibits an extraordinarily dense plexus formation and extends almost 3 times as far as the control.

steroids. Thus the very neuritic nature of this *in vitro* response, especially the temporal aspects, suggests that steroid-induced differences in neuritic (axonal)? growth patterns may play a role in the neurogenesis of sexual differentiation by so influencing dendritic differentiation and synaptic distribution of target neurons as to result in fundamentally different, gender-specific, patterns of neural organization. The *in vitro* observations would appear to provide a unifying concept or possible mechanism by which hormonal effects during neurogenesis could result in the observed structural differences in the adult brain. What all the described examples of androgen-dependent structural dimorphism seem to represent or share in common is that they may all be viewed as the end result of neuritic growth (dendritic branching patterns; synaptic terminals; synaptic organization) and thus, by definition, of neuronal differentiation and development (nuclear and nucleolar size; neuronal organelles); all parts of a whole—the neural circuit itself. Thus steroid-induced changes in neuronal metabolism during the critical period might express themselves morphogenetically in differences in the development or rate of development of the individual components of neural circuits, the end result of which could produce the observed instances of sexual dimorphism in the adult.

Finally, the *in vitro* observations also suggest that no pattern of sexual differentiation need necessarily be intrinsic to nervous tissue but that male and female patterns may *both* require active induction by steroid. Although alpha-fetoprotein in the rodent may sequester and greatly reduce the functional activity of the high perinatal levels of estrogen in both sexes, the actual extent of its inactivation is unknown. Perhaps, rather than being anhormonal, the genetic female exposed to such very low extracellular levels of estradiol during the critical period might develop a given pattern of neural organization. Intraneuronal aromatization of testosterone to estradiol, on the other hand, when present, could perhaps produce a more localized and concentrated estrogenic effect and the resultant stimulus to neuritic development might thus induce a different, or male, pattern of neural differentiation.

CONCLUSIONS

Despite the evidence of a neuroanatomic substrate for the gonadal steroid effects and, perhaps, for sexual differentiation, the fundamental neuronal biochemical processes by which androgen mediates its irreversible effects remain unknown. Ultimately they may be related to estrogen-induced changes in protein (Hudson *et al.*, 1970; Cavalloti and Bisanti, 1972) and/or nucleic acid (Salaman, 1974) biosynthesis.

Finally, it should also be noted that some studies suggest that, rather than producing structural changes in neural organization, neonatal androgenization may produce its effect, at least with respect to sexual behavior, by altering the responsiveness of the rat to hormonal and environmental influences (Beach, 1971). This has been shown by Clemens *et al.* (1969) in neonatally androgenized female rats who, after being first allowed to adapt to the testing situation, were shown to exhibit normal sexual behavior as compared with the non-adapted androgenized female.

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