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STEROID HORMONES AS MEDIATORS OF NEURAL PLASTICITY

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Summary—Steroid and thyroid hormone receptors are expressed in the developing brain and persist throughout adult life. They mediate a variety of effects on the brain, ranging from developmental effects of thyroid hormone and the process of sexual differentiation to the cyclic changes during reproductive cycles in adult female animals. This review summarizes data from the author's laboratory on three topics: (1) actions of extradiol and progesterone on the ventromedial nucleus of the hypothalamus in adult female and male rats, showing both the cyclicity and the consequences of brain sexual differentiation; (2) actions of estradiol on the cholinergic neurons of the basal forebrain of the female and male rat, reflecting the plasticity of the adult cholinergic system as well as sex differences which are developmentally programmed; and (3) diverse actions of estrogens, thyroid hormone and glucocorticoids on the morphology of hippocampal neurons. The review concludes by discussing the interactions between "organizational" (i.e. developmental) effects and the "activational" effects of steroids on the mature nervous system in relation to the environmental control of brain gene expression.

INTRODUCTION

The environment in which an individual grows up and lives has a major influence on the expression of the genetic characteristics with which he or she is endowed. This fundamental fact, which was unknown in the "nature/nurture" controversies of the early history of modern genetics [1], has emerged strongly from the advances in molecular and cellular biology. These investigations have revealed that the genome of virtually all living cells is continually in communication with the immediate environment of those cells and the more distant environment of the organism through the actions of numerous chemical messengers which can directly and indirectly influence gene expression. Among the most important of these messengers are the hormones, which are secreted in response to internal and external signals and act as coordinators of the body's response to a changing environment [2].

Circulating hormones of the gonads, adrenals and thyroid play an important role in modifying the structure and biochemistry of a variety of organs and tissues of the body, including the central nervous system [2]. These hormones act at the genomic level through protein receptors which are able to bind to specific DNA sequences that are located in the transcription–regulatory regions of particular genes [3, 4] (Fig. 1). The brain contains receptors for each of the 6 classes of steroid hormones as well as thyroid hormone and retinoic acid, and each receptor type has a unique and non-uniform distribution among the various structures and cell types of the brain [2]. Actions of these hormones are traditionally subdivided into "organizational" effects, referring to developmental actions which are permanent, and "activational" effects, which are transitory and reversible [2]. Traditionally, organizational effects have been thought to involve changes in neural structure and circuitry, whereas activational effects have been thought to involve changes in neurochemistry. Whereas these distinctions are supported by experimental data, they represent an over-simplification. In this article, we show

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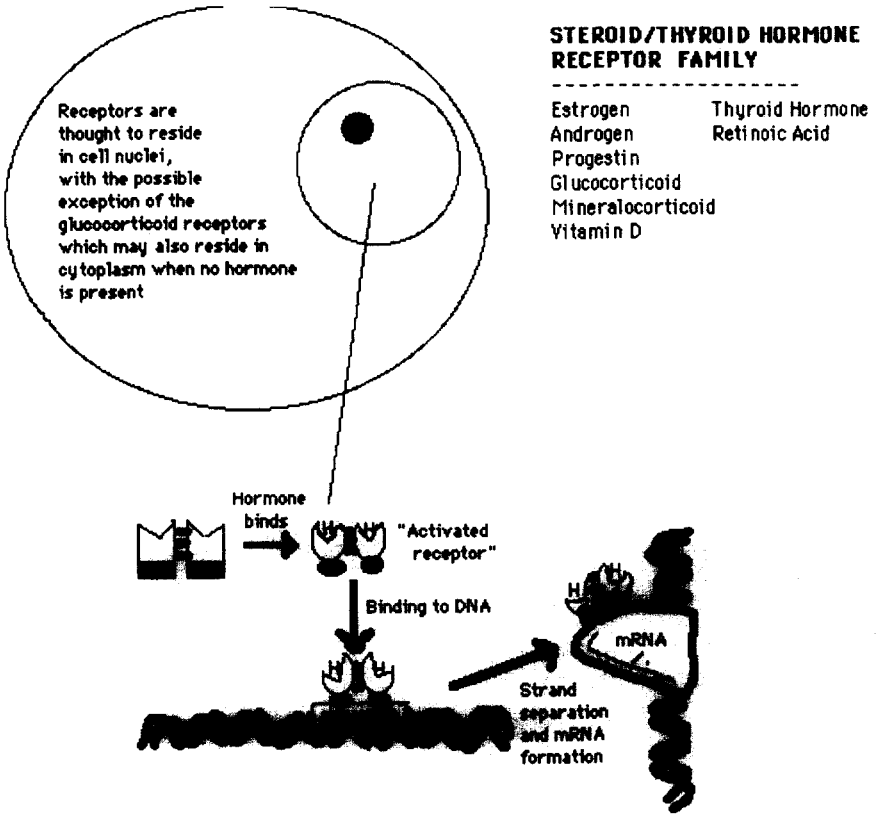

Fig. 1. Representation of the action of receptors of the steroid/thyroid hormone receptor family.

that activational effects do involve some structural changes in neurons, albeit transitory ones, and that organizational effects involve changes in the state of differentiation of hormone-sensitive neurons *vis-a-vis* how they respond to hormone "activation" in their mature state. We concentrate our discussion on two brain areas: the hypothalamus, the nexus of neuroendocrine regulation and vegetative behaviors and autonomic control; and the hippocampus, the nexus of cognitive and affective states.

ACTIVATIONAL EFFECTS OF GONADAL STEROIDS ON THE HYPOTHALAMUS

The hypothalamus is a control center for reproduction, which involves the coordination of sexual behavior with a neuroendocrine state that optimizes the chances for conception. In the female rat, the cyclic rise and fall of estradiol (E) primes and coordinates both LH secretion and sexual behavior. Studies using localized brain lesions and hormone implants have revealed that the ventromedial nuclei (VMN) of the hypothalamus are the key sites through which E and progesterone (P) act to induce feminine sexual behavior (lordosis). The actions of E and P in the VMN to induce lordosis involve RNA and protein synthesis, judging from the effects of inhibitors introduced into the VMN [5].

What are the key cellular events induced by E and P which cause lordosis to occur? Initial morphological observations indicated that VMN neurons respond very rapidly to E, within 2 h, and show evidence of massive increases in capacity for protein synthesis. These changes include decondensed chromatin in cell nuclei, larger nucleolus, more 28S ribosomal RNA, larger cell nuclear and cell body diameter [6]. Stimulated by the work of Carrer and Aoki [7], showing increased synaptic density in the VMN 48 h after E treatment, we have observed that dendritic spines density is dependent on E-priming. Ovariectomy decreases dendritic spine density, and E replacement restores it within 72 h; moreover, spine density fluctates in the estrous cycle, with a peak on proestrus and a lower level on estrus and diestrus [8]. Thus one of the reasons for the massive protein synthesis induced by E may be the formation of new dendritic spines and synaptic contacts.

Another facet of E and P actions involves neurochemicals involved in synaptic transmission. E treatment induces a 4–5 fold induction of receptors for oxytocin in the VMN, and it also causes neuronal fibers ventrolateral to the VMN to become filled with oxytocin immunoreactivity [9]. The only other part of the body where E induces massive numbers of oxytocin receptors is the reproductive tract [10] (see Fig. 2).

So what is the significance of this induction? When P is given to E-primed female rats, oxytocin receptors are seen to spread from the VMN itself out into the lateral area where the oxytocin fibers are seen. P treatment also makes the E-primed female rat sensitive to oxytocin infusion into the VMN as far as inducing lordosis behavior. The part of the VMN which is most sensitive to the behavioral actions of oxytocin is also the area which shows the P-induced spread of oxytocin receptors [11]. We do not know what mechanism is involved in the P effect, whether it represents an actual movement of receptors within dendrites or whether it represents as activation of preexisting receptors in dendrites, but the actions of P are clearly instrumental in promoting oxytocin as a neuromodulator of lordosis.

E treatment also induces P receptors in the VMN and other brain regions, and this induction is essential for P to exert its triggering effects on the reproductive process, including lordosis behavior [12, 13]. These are undoubtedly other E actions on neurochemical and morphological features of VMN neurons, besides those mentioned above. Induction of

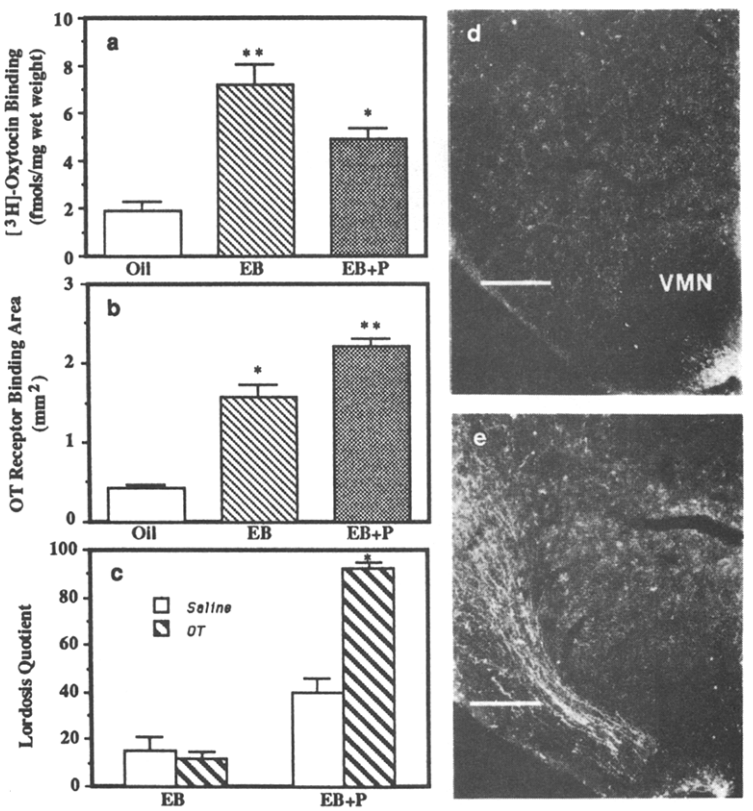

Fig. 2. Summary of experiments showing estrogen and progestin effects on oxytocin receptors in the hypothalamus of female rats and on feminine sexual behavior. (a) Specific oxytocin receptor binding in the VMN determined by quantitative receptor autoradiography. Estrogen priming induces oxytocin receptors in the VMN, and progesterone, applied after estrogen priming, further modifies receptor level; (b) area of specific oxytocin receptor binding on the VMN [mean +/− SEM; $n = 6$ for (a) and (b)]. Estrogen priming increases area occupied by oxytocin receptors in the VMN, and progesterone rapidly increases this area; (c) lordosis quotient for feminine sexual behavior. Thirty minutes before testing, ovariectomized females treated with estradiol benzoate (EB; $n = 10$) or EB plus progesterone ($n = 14$) were given bilateral infusions of 100 ng oxytocin in 1 μl of saline. Oxytocin stimulates feminine sexual behavior in estrogen-plus progesterone-primed female rats and not after estrogen priming alone ($P < 0.001$, Mann–Whitney U test); (d) oxytocin immunoreactive fibers are not seen lateral to the VMN in females not exposed to ovarian steroids; and (e) estrogen priming caused oxytocin immunoreactive fibers to become apparent lateral to the VMN. Scale bar = 50 μm. Reprinted from Ref. [9].

mRNA for preproenkephalin is one such effect [14, 15]. The suppression of GABAa receptor binding [16] and monoamine oxidase activity [17] and enhancement of muscarinic cholinergic receptor [18–21] binding are other reported effects. Therefore the story of lordosis regulation in the VMN will be even more complex than we could have anticipated.

Sex differences

The VMN also undergoes organizational effects of testosterone during neonatal development in the rat. Developmental sex differences occur in the lordosis response, with males showing very little feminine sexual behavior after castration in response to E + P priming [22]. The E-primed male rat appears particularly insensitive to the activational effects of P [23, 24]. It is therefore very interesting that the induction by E-priming of P receptors in the VMN is deficient in male rats [25]. This deficiency does not occur if the defeminization of lordosis behavior is blocked neonatally in the male rat; moreover, female rats which are defeminized by neonatal hormone treatment show male-like deficiency of P receptor induction by E [26]. In light of these sex differences, it is particularly intriguing that male rats show the same induction of oxytocin receptors by E as do female rats [27]. In fact, testosterone appears to be as good an inducer of oxytocin receptors as E in the male [28]. Yet, there is one major sex difference, namely, that P fails to induce the spread of oxytocin receptors in E-primed male rats that it causes in E-primed female rats [29].

How can one effect of E show sex differences, whereas another effect of E does not? One possibility is that different cells are involved. Specifically, the oxytocin receptor induction is rather widespread within the VMN [9], whereas the neurons which show the sex difference in P receptor induction are scattered throughout the VMN [30]. However, the true story may be more complicated, in that estrogen up-regulation of preproenkephalin mRNA, which is widespread in the VMN, is present in females and not evident in males [G. Romano, Ph.D. thesis, Rockefeller University, 1988]. The question for future research is whether enkephalin and oxytocin receptor induction by E coexists within the same, or different, neuron populations in the VMN. If they reside in the same neurons then the explanation of the differential regulation must lie in factors within the neuron which differentially regulate gene expression. Another intriguing factor to be considered in future research is that the induction of dendritic spines by E in VMN neurons also shows a marked sex difference: E induces spines in females, reversing the effect of ovariectomy, whereas E suppresses spine formation in males, reversing the effect of castration, which is opposite to the effect of ovariectomy [31, 32]. Thus some VMN neurons appear to be regulated in opposite directions by the same hormone as a function of the state of sexual differentiation.

ACTIVATIONAL EFFECTS OF ESTROGENS ON THE BASAL FOREBRAIN CHOLINERGIC SYSTEM

Another action of E-priming is the induction of choline acetyltransferase (ChAT) activity and enzyme amount in the basal forebrain of the female rat [33, 34] (see Fig. 3). This effect involves increases in actual enzyme amount and results in increased ChAT activity in projection areas of basal forebrain neurons, namely, the hippocampus and cerebral cortex [35]. The

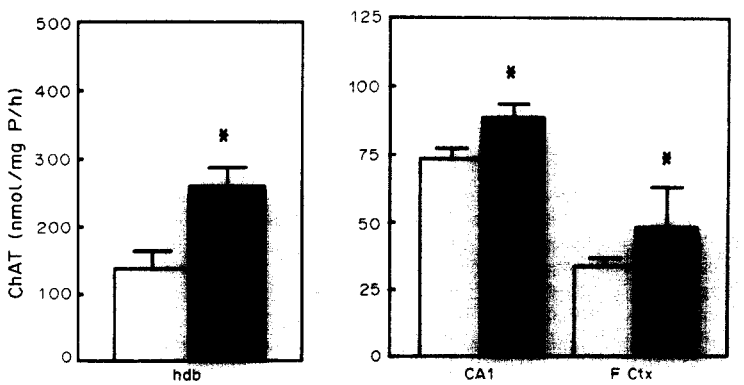

Fig. 3. Estrogen priming of ovariectomized female rats induces ChAT activity in the hdb and causes ChAT activity to increase in the CA1 field of the hippocampus, where heavy cholinergic innervation occurs, and in the frontal cortex. ■, estrogen-treated; □, control. Data from Ref. [35].

delayed appearance of ChAT activity in the hippocampus and frontal cortex suggests that newly-formed enzyme is transported to cholinergic terminals in these areas of the brain [35, 36] (see Fig. 3). Only the ChAT neurons of the horizontal limb of the diagonal band of Broca (hdb) appear to be sensitive to E; those in the septum and horizontal limb of the diagnonal band are not affected by E treatment. The relationship of this induction to events in the hippocampus will be discussed further below.

Sex differences

The induction of ChAT in the hdb by E shows a marked sex difference, in that it is absent in males and present in females [36] (see Fig. 4). Neonatal treatments which reversed the lordosis response to E + P in males and females did not alter the induction of ChAT by E in hdb, although they did have other effects on the development of the cholinergic system of the diagonal band of Broca [37]. These results thus lead us to believe that the septal–basal forebrain cholinergic system of the rat is sensitive to the activational and organizational effects of gonadal hormones. As will become evident below, thyroid hormone also has important developmental effects on this system which are dependent on sex as well.

ORGANIZATIONAL AND ACTIVATIONAL EFFECTS PF THYROID, GONADAL AND ADRENAL STEROIDS ON THE HIPPOCAMPUS

Because of the effects of E on basal forebrain cholinergic projections to the hippocampus, we turn our attention to this intriguing

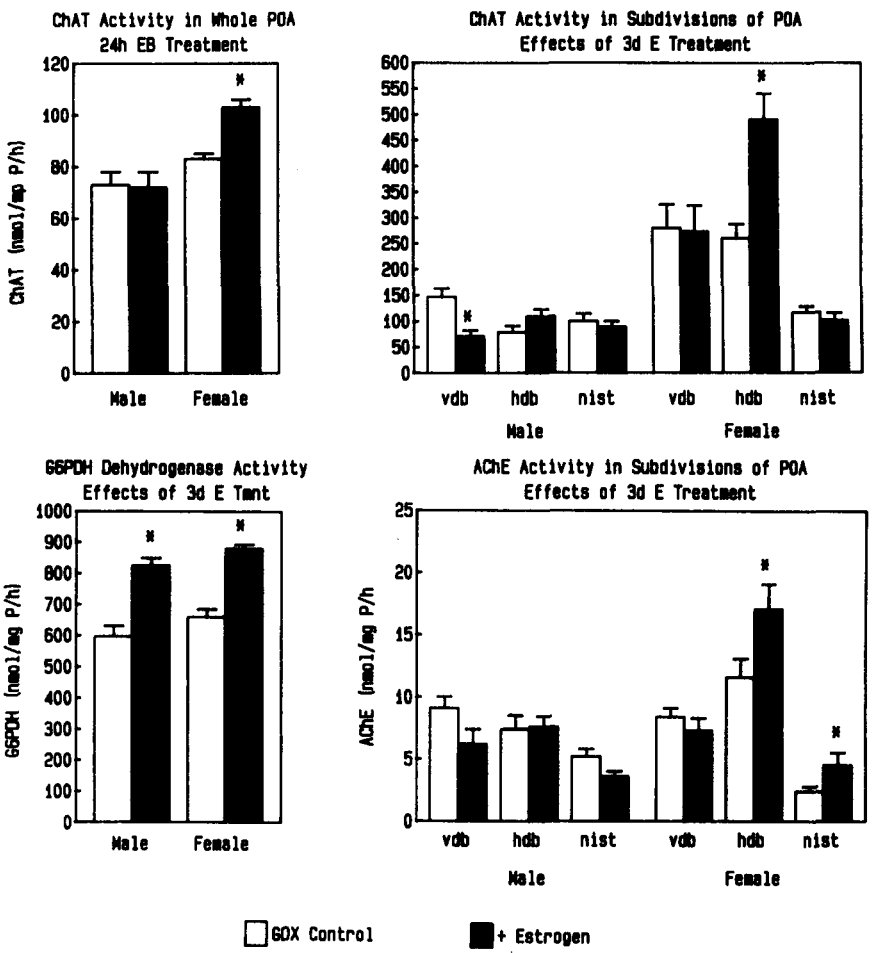

Fig. 4. ChAT activity is induced in the whole preoptic area (POA) and in the hdb of female rats, but not in males. Note that estrogen treatment is sufficient to induce glucose-6-phosphate dehydrogenase (G6PDH) activity equally in the male and female pituitary. Estrogen treatment appears to have a general effect in the hdb on cholinergic neurons, in that it also increases acetylcholinesterase (AChE) activity. Note that there are small estrogen effects in males to decrease ChAT in the vertical limb of the diagonal band of Broca (vdb) and to increase AChE activity in the bed nucleus of the strial terminalis (nist). Data from Ref. [36].

and enigmatic structure. The hippocampus is intriguing because of its highly organized, laminar neuroanatomy and it is enigmatic because its functions are so broad and difficult to specify. As part of the limbic system of the brain, the hippocampus is important in the affective state of the organism. It is also important in learning and retrieval of learned information. Because of its dual involvement in cognition and emotion, the hippocampus can be regarded as a place where these two neural activities come together and interact. Therefore it is not so surprising that the hippocampus is also a neural locus of sensitivity to hormones of the thyroid, adrenals and gonads (see Fig. 5).

Thyroid hormone

Some of these hormone effects on the hippocampus are seen developmentally as long-lasting organizational effects, whereas others are seen at maturity as activational effects. Thyroid hormone effects on hippocampal development are produced with very brief exposure of the new born rat to excess thyroid hormone [38]. Neurons in the CA3 subfield of neonatally thyroid hormone-treated rats become larger and more highly branched, and these effects persist in adulthood and are found in both sexes; the effects are specific in that the CA1 subfield is not markedly altered [38].

Sex differences

There is a sex difference in morphology of neurons in the CA3 subfield, which persists in the thyroid hormone-treated rats, indicating that the actions of gonadal steroids and thyroid hormone are largely independent for this brain area [38]. However, the same neonatal thyroid hormone treatment increases cholinergic enzymes and alters the morphology of cholinergic neurons of the basal forebrain and septum in male rats but has relatively little effect on females, even as it produces relatively similar effects in males and females on cholinergic neurons [39]. This developmental difference may be explained by sex differences in the rate of maturation of cholinergic neurons of the septum and basal forebrain [40], which is evidently independent of what happens in the hippocampus.

Adrenal steroids

The hippocampus is also sensitive to circulating glucocorticoids and contains two receptor systems for adrenal steroids [41]. One receptor system appears to respond largely to the diurnal variation in glucocorticoids, whereas the other receptor system responds to stress-induced glucocorticoid secretion. Adrenal steroids have a great variety of activational effects on the hippocampus and other brain regions

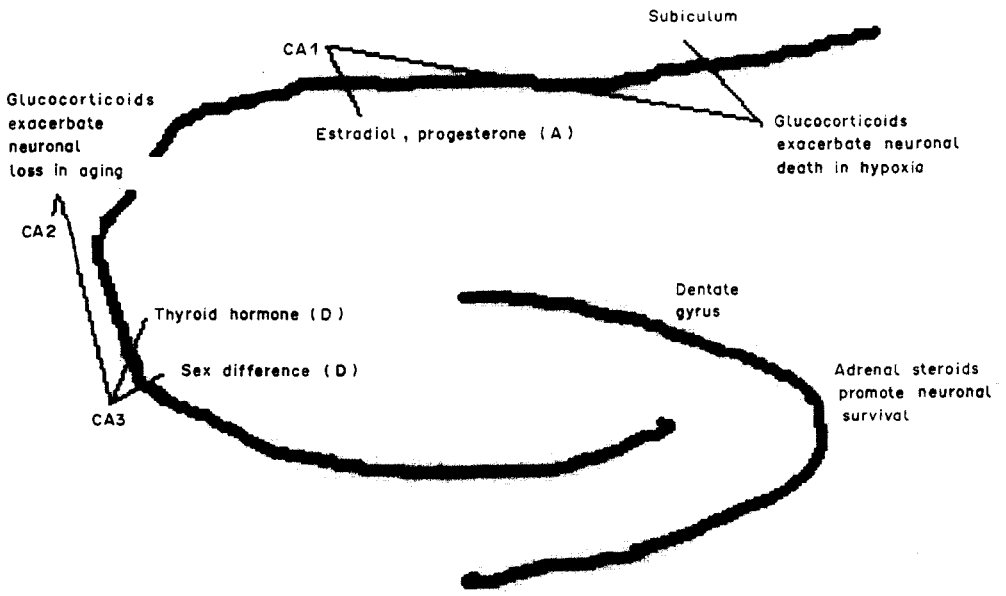

Fig. 5. The hippocampus is sensitive to a variety of hormones during development (D) and in adult life (A). Hormone effects alter neuronal size and shape, induce spines on dendrites, potentiate neuronate loss in aging and hypoxia and protect some neurons against death. Some neurons show sex differences in morphology and also reveal the long-lasting effects of neonatal hyperthyroidism. See text for details.

which affect neurochemistry and structure. Among the most puzzling and intriguing are the actions of adrenal steroids on the morphology and survival of hippocampal neurons. Glucocorticoids potentiate loss of neurons in the CA1 subfields associated with transient ischemia [42] and they potentiate loss of neurons in the CA3 subfield associated with aging [32–45]. Surprisingly, the dentate gyrus is affected in the opposite direction by glucocorticoids. Adrenalectomy (ADX) results in increased pyknosis of dentate granule neurons within as little as 3 days and neuronal size and branching patterns are reduced by ADX [46]. Both effects, which are prevented by low doses of glucocorticoid replacement, imply a trophic and life-sustaining influence of adrenal steroids. There is a reported, long-term affect of ADX to cause complete loss of the dentate gyrus in rats which are ADX for up to 3 months [47]. This effect is also prevented by corticosterone therapy [47], but it is peculiar why it only occurs in a sub-population of about 65% of the ADX rats, which also show signs of reduced serum Na and K and poor weight gain [47]. Clearly, other variables such as the presence of accessory adrenal tissue [48] may be involved in this rather dramatic effect of ADX.

As noted, pyramidal neurons of CA1 and CA3 fields of Ammon's horn undergo destruction in part due to the actions of glucocorticoids. The destruction in CA3 may be related to the dentate gyrus through activation of the mossy fiber system which projects from the granule neurons to dendrites of the CA3 pyramidal neurons. Daily administration of corticosterone to rats over 21 days results in shrinkage and debranching of the apical dendrites of CA3 pyramidal neurons, which receive the heaviest mossy fiber input [49]. This may reflect the activation of mossy fiber input by elevated glucocorticoids, and this would be consistent with the positive effects of glucocorticoids on granule neuron survival noted above.

The destructive effects of glucocorticoids on pyramidal neurons appear to be the result of interactions of glucocorticoids with other neurochemical system that affect neuronal survival, namely, excitatory amino acids and calcium [50]. One key action of glucocorticoids may be inhibition of glucose transport [51], which would have the effect of reducing available energy supplied for maintaining ion balance and sequestering calcium mobilized by the actions of excitatory amino acids [45, 50]. It remains to be seen whether the positive, trophic effects of glucocorticoids on the dentate gyrus involve any of the same mechanisms.

Estrogens

The hippocampus contains estrogen receptors which are found in all cell fields but in relatively low concentrations [52]. Estrogen receptor immunoreactivity and estrogen effects on gene expression are also reported in the hippocampus [53]. The CA1 regions show a low level of estrogen induction of progestin receptors [54], which is reminiscent of the hypothalamus (see below). It is therefore particularly interesting that the CA1 region is the site of highly localized effects of E-priming in adult female rats which induce spines on primary dendrites [55]. This effect of E is potentiated by as little as 4 h of priming with P; moreover, spine density fluctuates during the estrous cycle, with a peak on the day of proestrus when E priming is at its peak [C. Woolley, unpublished]. Apical dendrites on CA1 neurons show a more pronounced effect than basilar dendrites, and CA3 neurons and dentate granule neurons show no spine induction by E [55]. This pattern of effect is consistent with the pattern and intensity of cholinergic innervation of the subfields of the hippocampus [56], and it suggests one possible explanation, namely, that the spine induction by E is a postsynaptic correlate of the E regulation of ChAT activity and perhaps other properties of hdb neurons in the basal forebrain (see above). Various mechanisms might be involved, including actions of E on both hdb and CA1 neurons or actions of E only on CA1 neurons, with hdb neurons responding to altered production of trophic factors like NGF which are produced in high levels in the hippocampus [57]. As far as sex differences in the spine inducing effects of E, there is so far no data for males exposed to E, but one might expect there to be a difference in view of the lack of E effects to induce ChAT in the hdb of male rats [36].

CONCLUSIONS—INTERRELATIONS BETWEEN ORGANIZATIONAL AND ACTIVATIONAL EFFECTS

Steroid and thyroid hormone receptors are expressed in developing neural tissue and persist throughout adult life [2]. These receptors mediate both the organizational and activational effects of these hormones, and we have presented examples of both types of effects. In

some cases, we have seen evidence of interactions between the two types of effects. For example, the induction by E of lordosis behavior, progestin receptors and dendritic spines in the VMN is dependent on the state of sexual differentiation of the VMN; the same is true for the effects of E to induce ChAT in hdb. Another example is the effect of thyroid hormone on the septal–basal forebrain cholinergic system, which is different in males and females, suggesting an interaction of thyroid hormone actions with the state of differentiation of the cholinergic system as it is influenced by the developmental effects of gonadal steroids.

We have seen other effects, such as those of E on dendritic spines in CA1, which have not yet been explored for sex differences. Moreover, the actions of glucocorticoids on hippocampal neuronal survival have not yet been explored in relation to the sex of the animal or the thyroid hormone state during early development or adult life. Furthermore, our information is incomplete as to possible activational effects of thyroid hormone on hippocampal or hypothalamic neurochemistry or morphology, but it appears likely that there will be interesting effects [58–62].

What is the overall functional significance of these interactions between hormonal systems which have long-term effects via gene expression on brain structure and neurochemistry? The basic features of the brain are determined by information contained within the genome which is expressed during development and adult life. As we stated in the Introduction, environmental control of gene expression through the endocrine system is extremely important for shaping the brain and body to the demands of the environment and sometimes for the occurrence of disease processes. For example, the concordance of genetically-based diseases such as familial Alzheimer's disease between genetically-identical twins is only 50% [63], which means that the environment in which each twin grows and lives plays a powerful role in whether the disease will be expressed. There are undoubtedly many normal traits which also show such environmental influences, which means that individual differences in brain function and behavior [64] can be attributed in part to the cascade of environmental influences during early development which interact with and determine the impact of environmental factors in adult life.

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