J. Steroid Biochem. Molec. Biol. Vol. 40, No. 1–3, pp. 1–14, 1991
Printed in Great Britain.

0960-0760/91 $3.00 + 0.00

STEROID AND THYROID HORMONES MODULATE A CHANGING BRAIN

BRUCE S. MCEWEN,* HECTOR COIRINI, ANITA DANIELSSON, MAYA FRANKFURT, ELIZABETH GOULD, SCOTT MENDELSON, MICHAEL SCHUMACHER, ANNABELL SEGARRA and CATHERINE WOOLLEY

Laboratory of Neuroendocrinology, Rockefeller University, 1230 York Avenue, New York, NY 10021 U.S.A.

INTRODUCTION

Steroid hormones have been linked experimentally to behavior and brain function since the publication by Berthold [1] of studies showing for the first time that the testes of the rooster secrete products which control aggressive, sexual and vocal behaviors. However, it has taken more than 100 years since Berthold's publication until we have begun to appreciate the complex nature of steroid actions on any tissues of the body, including the complex steroid control of brain function and behavior. Advances in investigating the mechanism of steroid hormone action required technological developments such as tritiated steroids and recombinant DNA techniques, whereas progress in finding out how steroids affect the brain was delayed by the complexities of the brain and by the diffuseness of the neural steroid hormone receptors within it. Successful advances in elucidating steroid effects on the brain during the past 2 decades are due in large part to the enormous strides in neuroscience research and techniques. This article will review our current concepts of how steroid and thyroid hormones affect the structure and function of neural tissue, after first tracing some of the major historical developments in this field.

HISTORICAL PERSPECTIVES

The isolation, chemical identification and synthesis of steroid hormones was accompanied by major advances in understanding basic processes associated with reproduction and stress [2, 3], and yet it was not until the 1960s with the introduction of tritiated steroids [4, 5] that major progress occurred in understanding how steroid hormones affect cells by modulating gene expression via intracellular steroid receptors [6, 7]. The recent cloning of steroid hormone receptors [8, 9] provided evidence of their common structural homology as well as their homology to the thyroid hormone/retinoic acid receptors, and it helped to highlight their significance as DNA-binding, gene regulatory proteins. Early indications that steroids exert rapid effects on cell membranes, e.g. as anaesthetics [10, 11] were not aggressively studied, with several exceptions, until reports emerged of their ability to alter Ca ion levels [12, 13] and to interact specifically with the chloride channel of the GABAa–benzodiazepine receptor [14–17]. Now, a more balanced view of steroid action [17] acknowledges both genomic activation and cell membrane effects which sometimes work in concert to affect cellular processes, as will be discussed below.

As far as the brain and behavior are concerned, investigations during the 1930s–1950s made abundantly clear that reproductive and, to a lesser extent, stress-related steroids affect behavioral states of a wide variety of animals. The pioneering work of the late Frank Beach [18], W. C. Young [19] and Daniel Lehrman [20] established a field of research known as "hormones and behavior", related to physiological psychology and ethology, in which the basic characteristics of hormonal influences on mating, vocalization, aggressive and defensive behaviors were established. Of particular importance during this time was establishing that the higher vertebrate brain undergoes a period of sensitivity to gonadal steroid, which influences sexual development and differentiation. The recognition that there was sexual differentiation of neuroendocrine function [21]

Proceedings of the VIIIth International Congress on Hormonal Steroids, The Hague, The Netherlands, 16–21 September 1990.
*To whom correspondence should be addressed.

and behavior [22] led subsequently to neuroanatomical and biochemical studies which show that the brain itself undergoes sexual differentiation [23–25].

Introduction of tritiated steroids made possible the discovery of intracellular steroid receptor proteins in target tissues such as the uterus, liver, kidney and male accessory sex glands [6, 7, 26, 27]. Investigations of the brain soon followed and revealed sites within the brain of "uptake and retention" of steroid hormones. Estrogens were the first studied [28–30], followed by androgens [31]. Our own initial work with tritiated glucocorticoids revealed "uptake and retention" of tritiated corticosterone by the hippocampus, a rather unexpected locus [32]. The next step involved demonstrating the presence of cytosol steroid receptors and cell nuclear retention of injected ^3H steroid. This was accomplished first for estrogens [33] and subsequently for glucocorticoids [34–38].

The most powerful technique at the time for mapping steroid receptors in the brain was steroid autoradiography, which was used effectively first for estrogens and androgens, then for glucocorticoids and later for progestins, mineralocorticoids and vitamin D as well as thyroid hormone. See Table 1. Subsequent work has also employed steroid receptor antibodies for the receptor protein and cDNA or cRNA probes for the mRNA. Each hormone receptor has a somewhat unique pattern of distribution within the brain and pituitary gland, and this information has provided the basis for studies of the chemical characteristics and functional role of hormone-concentrating cell groupings. This is the focus of the remainder of this article. We shall concentrate on two regions of the brain: the hippocampus, which is a site of some of the most interesting of the cellular effects of steroids and thyroid hormone and the basal hypothalamus, in which we can study the combined genomic and non-genomic actions of reproductive hormones as they act to turn on sexual behavior in the female rat by a process which is sexually dimorphic.

Table 1. Mapping of steroid receptors in the brain by three methods

Steroid autoradiography
- Estrogens: Michael [39]; Stumpf [40]; Pfaff [41]; Attramadal [42]; Warembourg [43];
- Androgens: Pfaff [41]; Sar and Stumpf [44]
- Progestins: Sar and Stumpf [45]; Warembourg [46]
- Glucocorticoids: Gerlach and McEwen [47]; Stumpf [48]
- Mineralocorticoids: Birmingham *et al.* [49]; Ermisch and Ruhle [50]
- Vitamin D: Stumpf *et al.* [51]
- Thyroid hormone: Dratman *et al.* [52]

Immunocytochemistry
- Estrogens: Cintra *et al.* [53]; Blaustein and Turcotte [54]
- Progestins: Blaustein *et al.* [55]; Warembourg *et al.* [56]
- Glucocorticoids: Fuxe *et al.* [57]

In situ hybridization
- Estrogens: Simerly [58]
- Androgens: Simerly [58]
- Glucocorticoids: Aronson *et al.* [59]; Herman *et al.* [60]
- Mineralocorticoids: Arizza *et al.* [61]; Herman *et al.* [60]
- Thyroid hormone: Bradley *et al.* [62]

Three methods have been used to histologically reveal steroid hormone receptors in the nervous system.

STUDIES OF THE HIPPOCAMPUS AS A TARGET OF STEROID AND THYROID HORMONES

The hippocampus is a part of the limbic system of the brain and is also involved in the learning and recall of information [63]. The structure of the hippocampus is particularly orderly and attractive for neurophysiologists and neuroanatomists. Our attention was originally directed to the hippocampus by our finding that glucocorticoids are taken up and retained by neurons in the hippocampal formation of the rat [47] and rhesus monkey [64]. Subsequent work has begun to reveal the functional significance of adrenal steroid receptors in the hippocampus as well as the fact that the hippocampus is sensitive to estrogens, thyroid hormone, glucocorticoids in adult life and to thyroid hormone and the process of sexual differentiation during perinatal development.

Glucocorticoids

Besides regulating glial cell enzymes, several types of neurotransmitter receptors and calcium-calmodulin dependent adenylate cyclase activity in the hippocampus [65], glucocorticoids play an important role in neuronal survival under conditions of high and low hormone levels [66]. It was first observed that multiple injections of glucocorticoids or ACTH to guinea pigs over 3 weeks could cause hippocampal neuronal loss [67]. Subsequently, this was shown in rats. Moreover, it was shown that 12 weeks of daily corticosterone administration produces a pattern of cell loss within the hippocampal formation which mimics the loss of neurons seen with aging, viz. greatest loss in the CA3 region of Ammons horn [68]. Age-related neuronal loss in the hippocampus was attenuated by adrenalectomy in midlife [69].

We have recently found that a much shorter, i.e. 21 day, course of corticosterone treatment causes atrophy of apical dendrites of CA3 pyramidal neurons and no change in CA1 pyramidal neurons or dentate gyrus granule neurons [70]. The vulnerability of CA3 pyramidal neurons

during aging and as a result of glucocorticoid treatment made us curious as to a possible anatomical basis. Since CA3, and not CA1, pyramidal neurons receive heavy innervation from the dentate gyrus via the mossy fiber system, we examined effects of glucocorticoids on granule neurons of the dentate gyrus [71]. Whereas CA1 and CA3 pyramidal neurons are vulnerable to hypoxia, the effects of which are exacerbated by glucocorticoids, granule neurons do not appear to be vulnerable to this treatment. Rather, the absence of adrenal steroids resulting from adrenalectomy causes granule neurons to begin to die within 3–7 days, without noticeable effects in the pyramidal neuron population or on cerebellar granule neurons [71]. Some rats show massive loss of the entire dentate gyrus 2–3 months after adrenalectomy [72]. Steroid replacement at the time of ADX prevents this cell loss [71, 72]. Thus granule neurons appear to be positively dependent for their survival on glucocorticoids, which also help maintain normal granule neuron size and dendritic branching.

How does this relate to the CA3 pyramidal neurons? Stimulation of the dentate gyrus repeatedly via the perforant pathway causes damage to CA3 pyramidal neurons [73]. Moreover, kainic acid damage to CA3 pyramidal neurons is dependent on an intact mossy fiber system [74]. Because the effects of glucocorticoids on kainic acid damage and hypoxic damage appear to involve potentiation of the damaging actions of excitatory amino acids, it appears likely that repeated glucocorticoid administration may exacerbate the effects of excitatory amino acids produced by the mossy fiber system, Fig. 1. The mechanisms of this

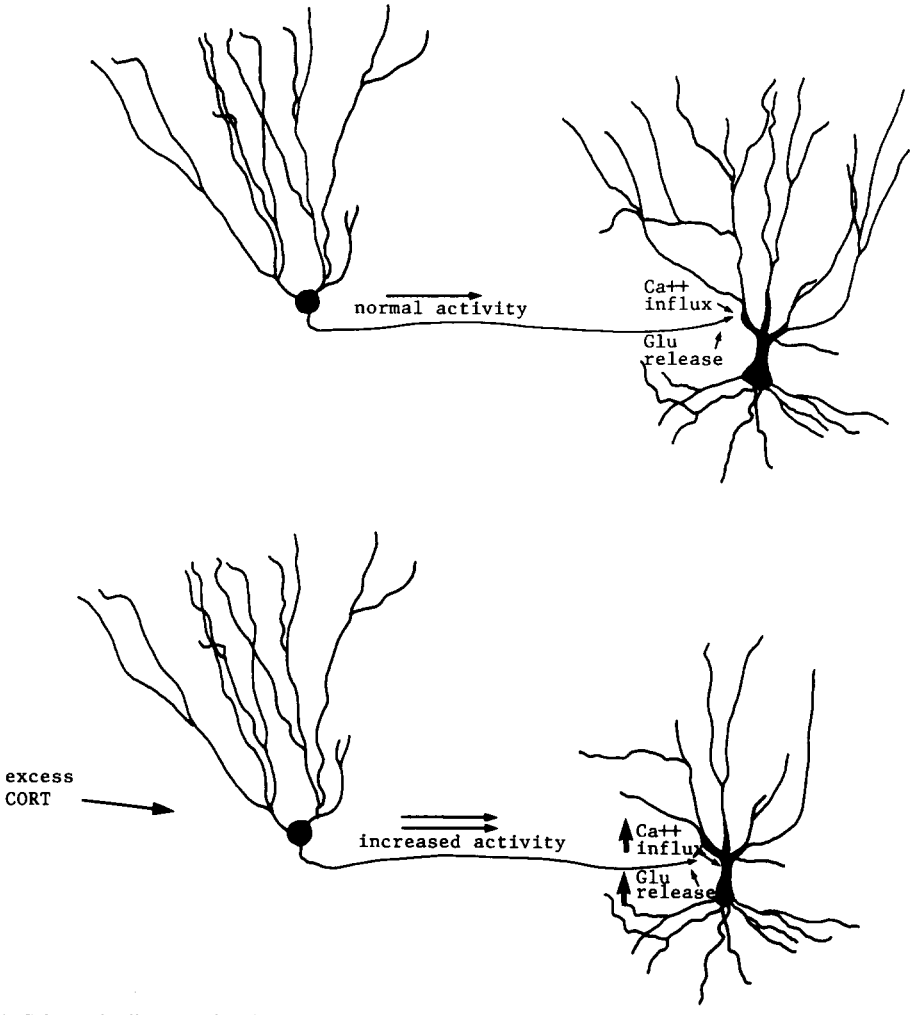


Fig. 1. Schematic diagram showing the postulated influence of the dentate gyrus mossy fiber system on the apical dendrites of CA3 pyramidal neurons, in which glucocorticoids enhance the mossy fiber system by positively influencing dentate granule neurons and exacerbate actions of excitatory amino acids on the dendrites of the CA3 pyramidal neurons.

damage are complex and beyond the scope of this review but are discussed extensively elsewhere [66, 75]. Since the dentate mossy fiber pathway is a major input to the pyramidal neurons of the hippocampus, it represents an access route for behavioral arousal and repeated stress to potentially affect hippocampal neuronal morphology. Recent studies of repeated social stress in vervet monkeys showing damage and neuronal loss in CA3 neurons supports this notion [76].

Estrogens

Estrogen treatment of ovariectomized female rats causes increased density of spines on the apical dendrites of CA1 pyramidal neurons [77]. There is also a cyclicity of spine density on these dendrites with the estrous cycle of the intact female rat [78]. CA1 neurons contain more estrogen receptors than other hippocampal cell fields as shown autoradiographically [79] and also by the presence of estrogen-inducible progestin receptors [80] and by the estrogen-induction of increased GABAa receptor binding [81]. Yet the asymmetry of the estrogen-induced increase in spine density of apical vs basal dendrites implies that afferent input may be involved [77]. One candidate is cholinergic innervation from the basal forebrain. This cholinergic innervation is under estrogenic influence, as shown by the induction and apparent transport of choline acetyltransferase from the horizontal limb of the diagonal band of Broca to the CA1 region [82–84]. It is not clear at present whether this induction and the induction of spines on CA1 neurons represents a concurrent action of estradiol in the basal forebrain and CA1 or is the consequence of action in the basal forebrain alone, Fig. 2.

Developmental effects of thyroid and sex hormones

Whereas CA1 neurons are subject to reversible morphological and biochemical actions of estrogens, as described above, CA3 neurons are affected developmentally by both thyroid hormone and sex hormones. There is a sex difference in the number of primary dendrites and spine density on CA3 neurons which is maintained and even accentuated if male and female rats are treated on days 1,2 and 4 postnatally with 0.5 μg/kg triiodothyronine (T3) [85], Fig. 3. Both males and females show enhanced numbers of dendrites and spine density as adults as a result of this transient neonatal T3 treatment [85]. In contrast, treatment of adult rats with a similar T3 dose produces no changes in CA3 neuronal mor-

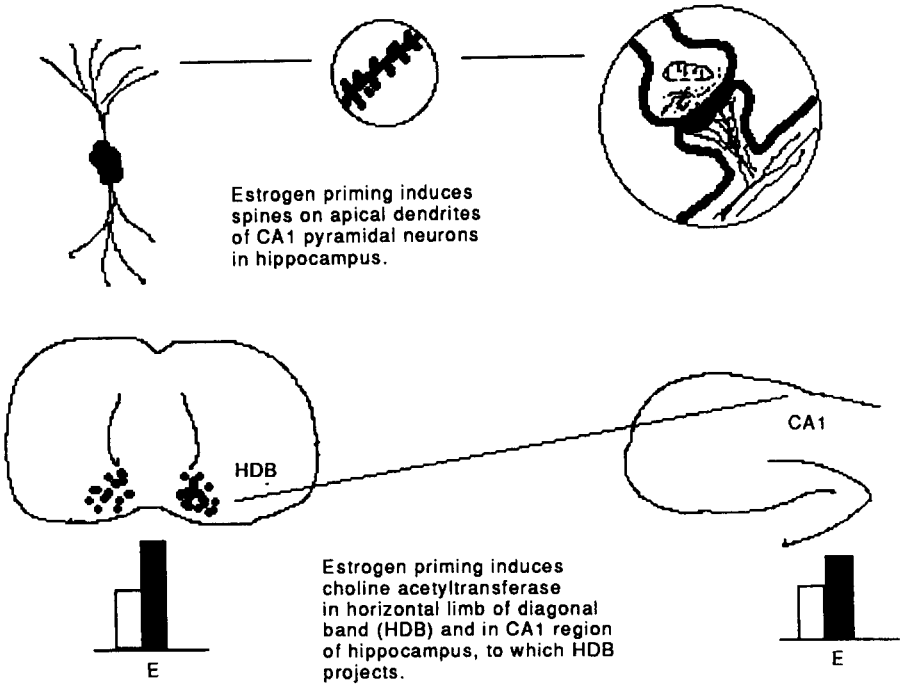


Fig. 2. Schematic diagram showing the coordinated actions of estradiol to induce cholinergic enzymes in the horizontal limb of the diagonal band of Broca (HDB) and in the CA1 region of the hippocampus, to which it projects and to induce spines on apical dendrites of CA1 neurons.

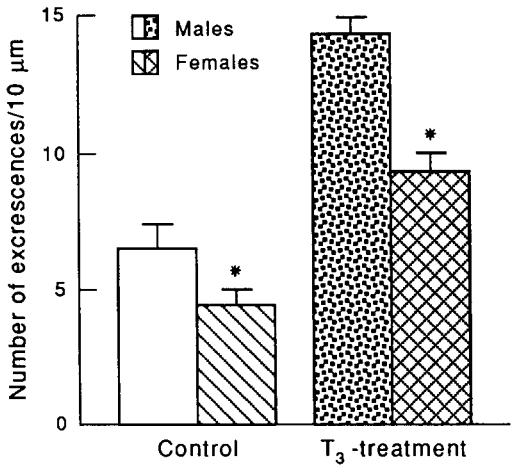


Fig. 3. Sex difference in and effects of transient neonatal hyperthyroidism on CA3 pyramidal neurons of the hippocampus. The figure shows the number of spines (excrescences) per 10 μm of dendritic length on apical dendrites of CA3 pyramidal neurons in both male and female euthyroid at birth and neonatally T3 treated rats. *Indicates significance at $P < 0.01$ from *posthoc* comparisons after a two-way ANOVA had shown no significant interactions between sex and treatment. Reprinted from [85] by permission.

phology but decreases spine density in CA1 pyramidal neurons [86].

Whereas neonatal T3 treatment produces similar qualitative changes in CA3 neuronal morphology in male and female rats, it produces differential effects on the basal forebrain cholinergic system [87]. T3 treated male rats show, as adults, increased choline acetyltransferase activity and increased density of cholinergic cell bodies in the septum, whereas T3 treated females do not show such alterations. Figure 4 shows the increased frequency of varicosities on cholinergic neurons in the medial septum of neonatally T3 treated male rats. It would appear that the different developmental time course of the cholinergic system in male and female rats is an important variable in its sensitivity to transient neonatal hyperthyroidism [87].

Neurons are not the only structures affected by transient neonatal hyperthyroidism [88]. T3 treatment at birth increases numbers of primary processes and size of astrocytes in the basal forebrain and hippocampus (Fig. 5).

The enhancement of CA3 neuronal morphology, astrocyte morphology and the septal–basal forebrain cholinergic system produced by transient neonatal hyperthyroidism is associated with poorer, rather than better, performance in spatial learning tasks and measurements of hippocampal long-term potentiation [89]. Errors and time to criterion are greater in rats treated neonatally with T3. Likewise, long term potentiation elicited *in vivo* by stimulating electrodes in perforant pathway and recording electrodes in dentate gyrus shows a higher threshold in T3 treated rats than in controls.

STUDIES OF HORMONAL CONTROL OF SEXUAL BEHAVIOR IN THE HYPOTHALAMUS AND PREOPTIC AREA

Ever since the work of Berthold, behavioral endocrinologists have been striving to understand how hormones bring about changes in behavior. Use of intracranial hormone implants showed that the brain is actually the site where hormones act to cause changes in behavior [90],

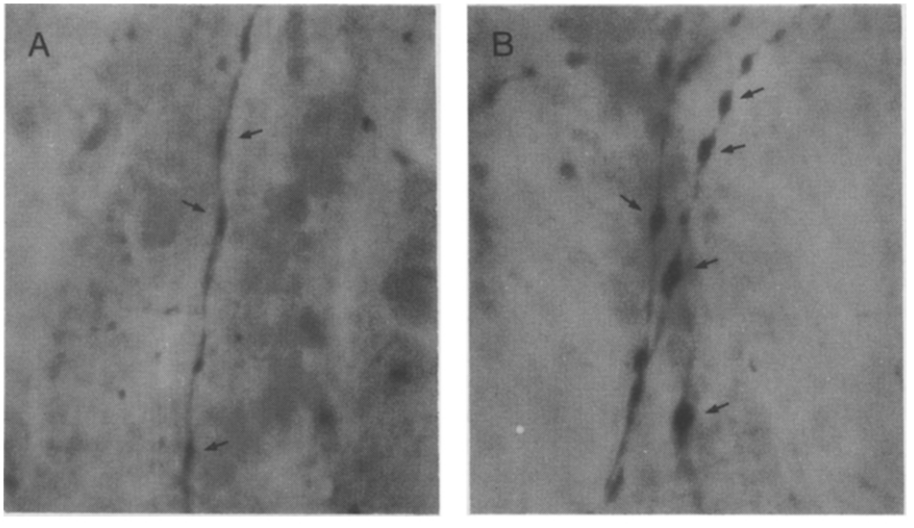


Fig. 4. Photomicrographs showing choline acetyltransferase immunoreactive fibers in the medial septum of male control (A) and male T3 treated (B) rats. T3 treatments were on days 1, 2 and 4 of neonatal life. Arrows indicate varicosities which are greater in size and frequency in T3 treated males (B) compared to controls (A). Females treated neonatally with T3 do not respond as dramatically. For details, see [87].

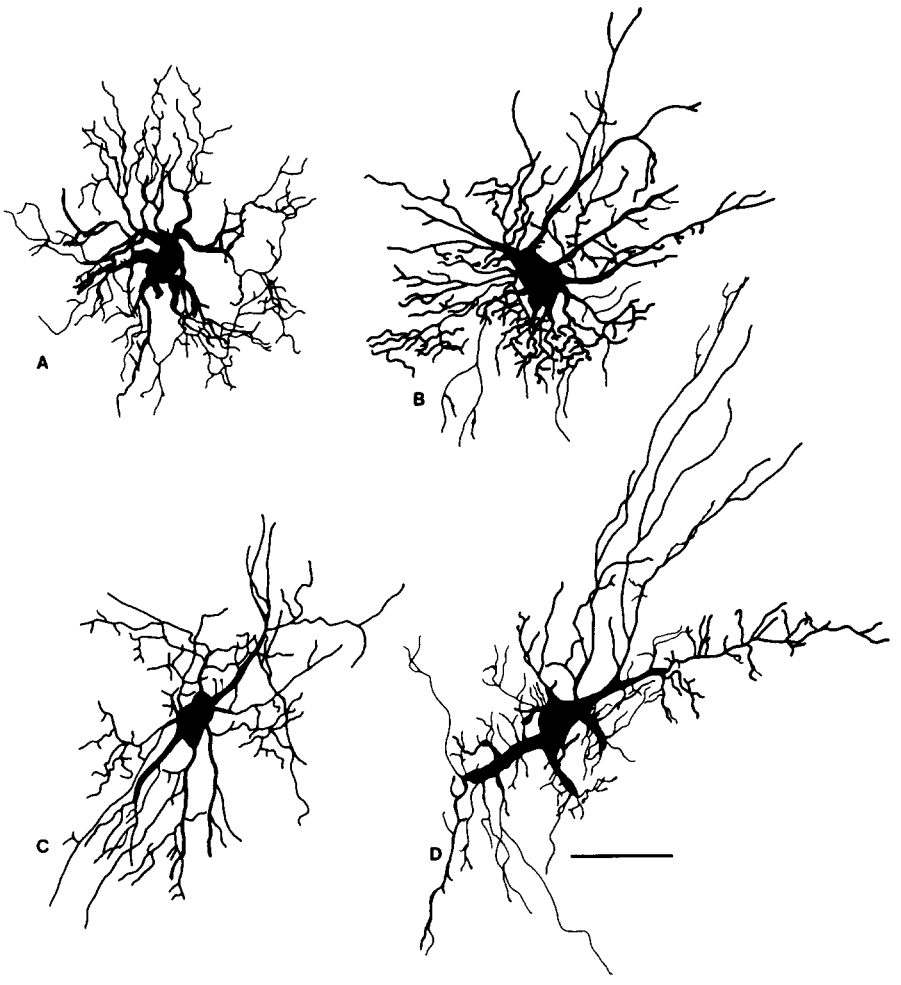


Fig. 5. Effect of transient neonatal hyperthyroidism on morphology of astrocytes. Camera lucida drawings of Golgi impregnated astrocytes show larger size and greater branching pattern of protoplasmic astrocytes (A, B) and fibrous astrocytes (C, D) in the nucleus basalis of neonatally T3-treated (B, D) rats compared to controls (A, C). Scale bar (——) in D equals 20 μm and applies to all drawings. From [88] by permission.

and the identification and mapping of steroid receptors in the brain (Table 1) provided a substrate for these actions. Much of the current work seeks to identify key hormone-regulated cellular events which are responsible for triggering the behavioral changes. Among the prime candidates are the hormonal induction of neurotransmitters and their receptors and of synaptic and dendritic structures, because chemical neurotransmission and the wiring of the brain would appear to be the most likely sites of regulation. We have been studying two systems in the brain; the preoptic area in relation to androgen dependent male sexual behavior in the rat and the ventromedial nucleus in relation to estrogen/progesterone regulated female sexual behavior in the rat. Each system allows us to examine a different aspect of the problem, such as the importance of hormone-changes in neurotransmitter receptor sensitivity and synaptic connectivity, the interaction between genomic and non-genomic effects of steroids and the effects of sexual differentiation on key hormone-dependent responses.

Androgen actions on the preoptic area

The preoptic area plays an important role in mediating androgen effects on male sexual behavior in the rat and other species, and serotonin is an important neurotransmitter affecting this behavior. Contrary to the original notion that serotonin has only inhibitory effects on male sexual behavior, there is now evidence that serotonin receptor subtypes participate in facilitative as well as inhibitory effects of serotonin on male sexual behavior [91]. In particular, 5HT1A receptors facilitate male sexual behavior [91], whereas 5HT1B receptors appear

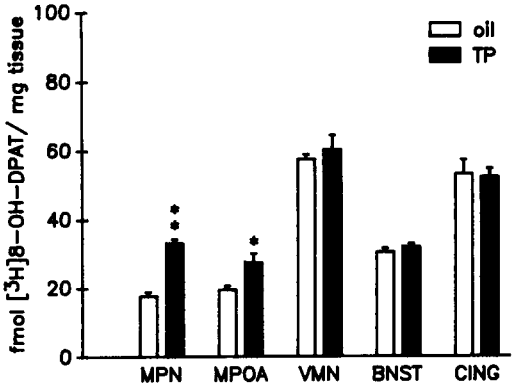


Fig. 6. Effect of androgen priming of density of 5HT1A receptors labelled by [^{3}H] 8-OH-DPAT in a number of brain regions, as determined by quantitative neurotransmitter autoradiography. Reprinted from [94] by permission.

to inhibit it [92]. On the other hand, blockade of 5HT3 receptors reveals increased levels of various social, including sexual, behaviors in rats [93]. We now have evidence that the type of androgen treatment which induces male sexual behavior increases 5HT1A receptor binding in the preoptic area and decreases 5HT3 receptors in amygdala [94]. Figure 6 shows the effect of androgen treatment of 5HT1A receptor binding in various brain regions. 5HT1B receptors are not affected by androgen priming (Mendelson, unpublished). However, prenatal exposure to ACTH, which decreases male sexual behavior in adulthood, specifically increases 5HT1B receptors in the male rat preoptic area without changing 5HT1A receptor density [95]. It would thus appear that developmental events and androgen priming vie with each other to determine the balance between receptors densities which mediate facilitative and inhibitory effects of a key monoamine neurotransmitter. Indeed, the densities of some 5HT1 receptor types appear to be regulated by hormones, more so than by serotonin itself [96].

Estrogen–progesterone actions on the ventromedial nuclei

The ventromedial nuclei (VMN) of the hypothalamus are the key hormone-sensitive structures which control female sexual behavior in the rat and other species [97], and the study of their neurochemical anatomy and plasticity in response to estradiol and progesterone has provided the best picture so far available of the complex cellular processes which are necessary to alter the behavioral state of an animal as well as the rate-limiting steps which are the subject of sexual differentiation.

The VMN of the female rat rapidly responds to estrogen priming by showing neuronal enlargement, nuclear and nucleolar enlargement and evidence of increased capacity for protein synthesis [98]. These changes take only a few hours and they lead over 24–48 h in increased levels of a variety of gene products which are important for altered cellular connectivity and function. Among these are induction of receptors for progesterone [99], induction of receptors for oxytocin as well as oxytocin itself [100], and induction of spines on the VMN dendrites as well as the synapses which contact the spines [101], Fig. 7.

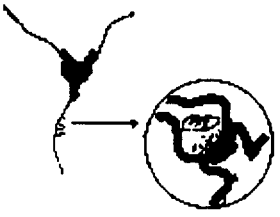
Spine density increases are accompanied by increased synaptic density in VMN [102], which indicates that there is new synapse formation. Spine density changes dramatically during the estrous cycle of the female rat, providing evidence of cyclicity of brain morphology in a mammalian species [101]. (The seasonal change in brain morphology of song-control nuclei in songbirds was the first example of this type [103].)

Progestin receptor induction by estradiol is closely correlated temporally and in terms of dose and duration of estrogen treatment with the conditions for turning on female sexual behavior, which requires sequential exposure to estradiol followed by progesterone [104]. The VMN is particularly sensitive in female rats to the rapid induction of progestin receptors, and progesterone implants in various brain regions reveal the VMN to be the only site where facilitation of sexual behavior will occur in the estrogen-primed animal [105].

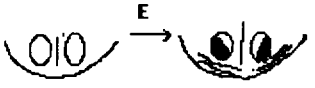
Estrogen induction of oxytocin receptors in the cell body region of the ventrolateral VMN is accompanied by two additional events, as summarized in Fig. 7: (1) induction of oxytocin immunoreactivity in fibers ventrolateral to where the receptors are being induced; presumably these fibers carry peptide transported from sites of synthesis in cell bodies in the paraventricular nuclear region of the hypothalamus and (2) a gradual spread of induced oxytocin receptors ventrolaterally toward the oxytocin immunoreactive fibers over 24–48 h after estrogen treatment [100, 106]. As shown in Fig. 8, this spread occurs in the posterior part of the VMN but not in the anterior part of this nucleus. Yet estrogen priming alone is not sufficient to induce female sexual behavior or to permit oxytocin infused into the VMN to facilitate the behavior; rather, progesterone

Neurons of the ventrolateral VMN contain estradiol (E) and progesterone receptors. These neurons have dendrites which project laterally into the basal hypothalamus.

E treatment induces rapid enlargement of VMN neuron soma diameter and cell nuclear size, as well as increased nucleolar size and more ribosomal RNA and rough endoplasmic reticulum.



One result of increased protein synthesis is the induction of spines on VMN dendrites, as well as to increase synaptic density. Changes in spine density are also seen during the estrous cycle in the female rat.



Another consequence of increased protein synthesis is the induction of progesterone receptors in ventrolateral VMN. E also induces oxytocin receptors and causes oxytocin immunoreactive fibers to appear lateral to the VMN.

Fig. 7. Schematic diagram showing the coordinated effects of estradiol in ventromedial hypothalamic neurons to induce spines on dendrites, progestin receptors and oxytocin receptors in cell bodies, and oxytocin immunoreactivity in fibers projecting to the ventromedial hypothalamus.

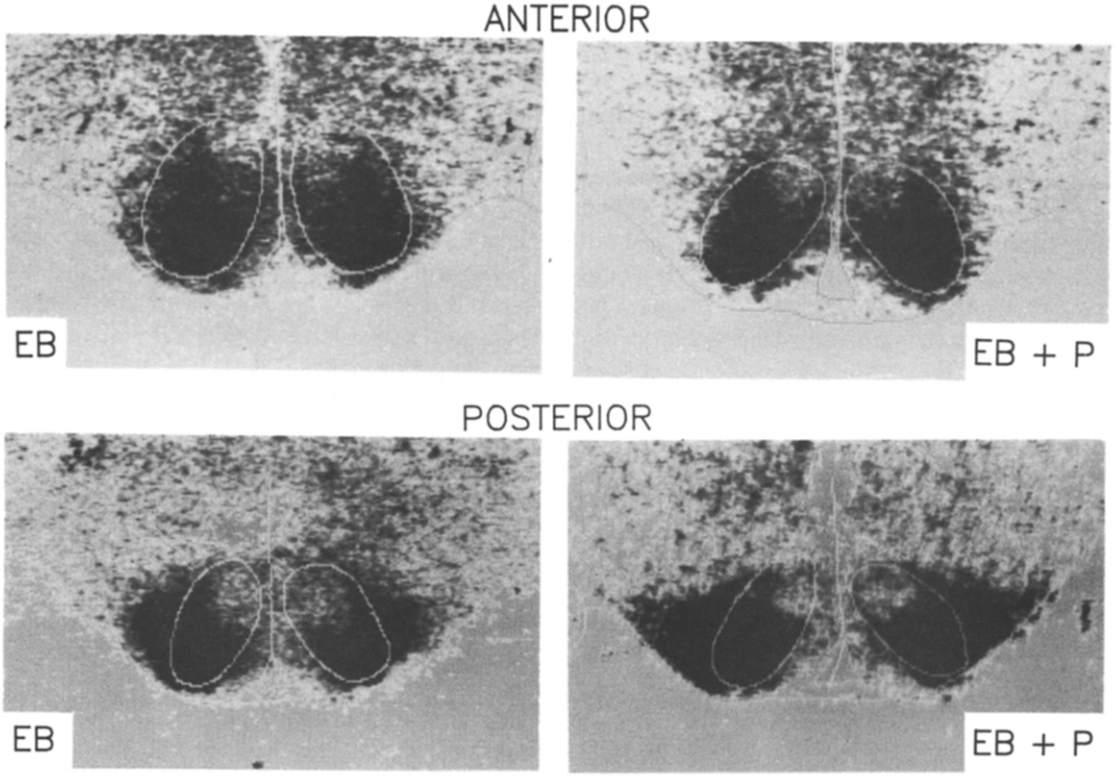


Fig. 8. Representative autoradiograms of oxytocin receptor binding in the anterior and posterior planes of the ventromedial hypothalamus of ovariectomized female rats treated with estradiol benzoate (EB) over 48 h (left side) or EB for 48 h plus progesterone for 4 h (EB + P, right side). The ventromedial nuclei outline was drawn from the histology by superimposing it on the autoradiographic image in the computer. The anterior and posterior planes of the VMN correspond to coronal plates 27–28 and 30–31 of the Paxinos and Watson [126] atlas. In the anterior VMN, oxytocin receptor binding was confined to the VMN and was not modulated by P, whereas in the posterior VMN the oxytocin receptor field extended laterally to the VMN and was increased by P treatment. From [106] by permission.

treatment for as little as 30 min–1 h is needed, and this treatment also induces a further spread of the oxytocin receptor field into the area where the oxytocin-immunoreactive fibers are located [100, 106]. This progesterone-induced spread occurs in the posterior VMN and not in the anterior region. The relationship of this effect to behavior is very striking, because oxytocin infusion is only effective in facilitating lordosis in the posterior VMN [106].

Perhaps the biggest surprise of all is that progesterone induction of the spread of oxytocin receptors can be replicated *in vitro* in VMN on sections which are previously frozen, desiccated and prepared for oxytocin receptor autoradiography [106]. The specificity of the progesterone effect favors progesterone over estradiol, cholesterol or 5α reduced metabolites of progesterone. In this regard, the specificity differs from the steroid specificity of interactions with the chloride channel of the GABAa receptor [16], but it may resemble that of membrane actions of progesterone to modulate LHRH release via a calcium-dependent process [107]. Thus it would appear that progesterone actions on the oxytocin receptor system and through it on lordosis behavior involve a non-genomic mechanism which is dependent on a prior genomic action of estradiol. The membrane effect of progesterone to induce the oxytocin receptor "spread" may involve a rapid translocation of receptors on the dendrites of the VMN neurons in which the receptor is induced, or it may involve the activation of oxytocin receptors from a low to a high affinity state [106].

It is important to note that the membrane actions of progesterone on the oxytocin receptor may be a necessary part of this hormone's actions on lordosis behavior, but they are not sufficient. Evidence using a protein synthesis inhibitor, anisomycin, to block progesterone-facilitated lordosis behavior indicated that a rapid and possibly genomic action of progesterone is also involved in the facilitation of lordosis in estrogen-primed female rats [108]. It is not known what gene products are involved in this regulation, only that the proteins appear to have a short half-life [108].

To avoid the impression that these events described above are the only ones occurring in the VMN after E treatment, it must be noted that there is also induction of the mRNA for enkephalin [109] and modulation of monoamine oxidase activity [110]. Other neurotransmitter receptors are also regulated: muscarinic cholinergic receptor density increases by about 30% after estrogen priming [111, 112], whereas GABAa receptor density decreases by about 40% [113, 114]. The GABAa receptor change is intriguing, because it also occurs in midbrain central gray (MCG) and arcuate nucleus (ARC); yet progesterone treatment reverses the decrease in VMN and MCG, which are part of the circuit mediated lordosis, but not in ARC, which is involved in regulating LH and prolactin secretion by the pituitary [114]. Moreover, as noted above, estrogen priming increases GABAa receptor binding in CA1 of the hippocampus [81]. What may explain these disparate effects is the distribution of isoforms of the GABAa receptor, whose subunit composition may reflect greater or lesser regulation by gonadal steroids in terms of gene expression and also local membrane effects.

The VMN and its various responses to estradiol and progesterone provide many insights into how sexual differentiation impacts on sexual behavior as well as brain structure and neurochemical responses to hormones. The VMN shows morphological sex differences, males having more axodendritic shaft and spine synapses than females, indicating more or at least different afferent input [115]. The male VMN is refractory to the actions of estradiol as far as dendritic spine induction [116, 117], muscarinic receptor regulation [118] and progestin receptor induction [119–122] are concerned, and yet it responds as equally well as the female to estrogen induction of oxytocin receptors [123]. However, progesterone-induced spread of oxytocin receptors is not evident in estrogen-primed, previously castrated male rats [124]. Future studies will need to determine if the characteristics which are sexually dimorphic in the VMN and those which are not are present in different cell types or are resident within the same cell types.

CONCLUSIONS

In this article we have reviewed recent work which illuminates the diverse ways in which steroid and thyroid hormone receptors are involved in modulating gene expression in the nervous system. Besides providing elegant examples of such modulation in specific neural structures and in relation to specific behavioral and neuroendocrine mechanisms, these recent studies have also made it evident that not all important or interesting effects of steroids

involve actions on the genome but rather include actions at the membrane level.

In addition to affecting the development of selected populations of neurons and glial cells during critical periods of perinatal development, these hormones also modify neuronal structure and chemistry in the mature nervous system. The most striking examples of these changes are the cyclic changes in dendritic spines during the estrous cycle of the female rat which have been found in the ventromedial hypothalamus and in the hippocampus. These phenomena indicate that the mature nervous system is more plastic than previously believed and suggest that there may be other reversible modifications of synaptic and dendritic structure within other endocrine cycles.

Just as the cyclic changes in neuronal morphology are prime examples of so-called "activational effects" of hormones resulting from natural endocrine cycles, the "organizational effects" of sex hormones leading to brain sex differences are also consequences of programmed changes in endocrine function. In this article, we have reviewed examples of heretofore unexpected sex differences in the CA3 neuronal morphology of the hippocampus as well as sex differences in the response of ventromedial nucleus neurons to progesterone. Sex differences in progesterone sensitivity at the genomic level arise from perinatal actions of testosterone acting via conversion to estradiol [122], but it is not yet known whether the membrane actions of progesterone on oxytocin receptor spread will follow the same rule. In the hippocampus, there is evidence for transient perinatal expression of both estrogen receptors and aromatase activity in moderate levels [125]. This provides one possible explanation for the origin of the sex differences in CA3 neuronal morphology, which must be developmentally determined since there are no known effects of sex hormone manipulations in adulthood which mimic the sex differences in CA3 neuronal morphology [77].

Whereas "activational effects" are inherently reversible, the "organizational effects" are largely irreversible, and yet they are expected within the normal framework of development and lead to the divergence of male and female patterns of brain function. In contrast, the irreversible effects of glucocorticoids to cause destruction of hippocampal neurons during aging and possibly also during certain types of stress may be called "disorganizational effects" which contribute to destruction and disregulation of brain function. Insofar as these changes are the result of "wear and tear" during natural cycles of glucocorticoid secretion, these effects may be viewed as part of a prolonged developmental process. However, insofar as these changes may be the result of certain types of stressful experience, they fall into a different category of hormone effect, one which is not cyclic or programmed but rather dependent on social interactions and external events which drive hormone output. Moreover, the destructive effects of glucocorticoids on CA3 pyramidal neurons may depend heavily on an input from the dentate gyrus and on the actions of neurotransmitters such as excitatory amino acids [66, 75]. It is the involvement of at least two chemical messengers, the excitatory amino acids and the glucocorticoids, which allows for the high degree of unpredictability of the final outcome and for possibly unique effects of particular behavioral states on the structure of the brain.

Another key feature of hormone action in the brain is the regulation of receptors of neurotransmitters. Estrogen induction of oxytocin receptors in the ventromedial hypothalamus appears to play an important role in facilitating female sexual behavior in the rat, and the induction by androgens of 5HT1A receptor in the preoptic area of the male rat may play a regulatory role in facilitating male sexual behavior. The actions of estradiol and progesterone on the oxytocin receptor system are particularly intriguing for several reasons and deserve considerably more detailed investigation. First, these actions appear to play an important role in turning on a behavioral response and they provide a first glimpse into some of the sophisticated and complex events that are involved in switching on reproductive behavior. It remains to be seen how the induction by estradiol of spines on dendrites and new synapses are related to the oxytocin system, and how estrogen effects regulate GABAa receptors, muscarinic cholinergic receptors, monoamine oxidase and preproenkephalin mRNA also fit in to the cascade of events which culminate in behavioral activation. A second important feature of the oxytocin system is that estradiol not only induces receptors for oxytocin but induces the neuropeptide oxytocin itself to appear in larger amounts in the ventromedial nuclei. The problem is that the receptors and the oxytocin are in different locations. The function of progesterone appears to be to bring them

together, and it does so at the membrane level either by activating preexisting receptors from a low to a high affinity state or by causing receptors to translocate along the dendrites of the VMN neurons in which they are induced by estradiol. This intriguing process is another example in a growing number of membrane actions of steroids, which include effects on the GABAa receptor chloride channel [14–17], on membrane mechanisms subserving release of LHRH and of dopamine [107] and on the mobilization of calcium [12, 13]. Future studies will need to examine which of these effects occur naturally as a result of progesterone itself or of metabolites of progesterone whose existence has been known for many years without any function [17] and will need to assess the relationship between these membrane actions and the genomic actions of the parent steroids via classical intracellular receptors.

REFERENCES

1. Berthold A. A.: Transplantation der Hoden. *Archs Anat. Physiol. Wiss. Med.* **16** (1849) 42–60.
2. Selye H.: The evolution of the stress concept. *Am. Scient.* **61** (1973) 692–699.
3. Young W. C. Sex and Internal Secretions. Williams and Wilkins, Baltimore (1961) p. 1609.
4. Glascock R. and Hoekstra W.: Selective accumulation of tritium-labelled hexoestrol by the reproductive organs of immature female goats and sheep. *Biochem. J.* **72** (1959) 673–682.
5. Gupta G. N.: The fate of tritium-labelled estradiol 17-beta in rat tissues. *Ph.D. Thesis*, Univ. of Chicago, Dissertation Abstr. Int. **34** (1972).
6. Jensen E. and Jacobson H.: Basic guides to the mechanism of estrogen action. *Rec. Prog. Horm. Res.* **18** (1962) 387–408.
7. Gorski J., Toft D., Shyamala G., Smith D. and Notides A.: Hormone receptors: studies on the interaction of estrogen with the uterus. *Rec. Prog. Horm. Res.* **24** (1968) 45–72.
8. Yamamoto K.: Steroid receptor regulated transcription of specific genes and gene networks. *A. Rev. Genet.* **19** (1985) 209–252.
9. Evans R.: The steroid and thyroid hormone receptor superfamily. *Science* **240** (1988) 889–895.
10. Selye H.: Correlations between chemical structure and the pharmacological actions of the steroids. *Endocrinology* **30** (1942) 437–453.
11. Holzbauer M.: Physiological aspects of steroids with anaesthetic properties. *Med. Biol.* **54** (1976) 227–242.
12. Godeau J., Schorderet-Slatkine S., Hubert P. and Baulieu E-E.: Induction of maturation in *xenopus laevis* oocytes by a steroid linked to a polymer. *Proc. Natn. Acad. Sci. U.S.A.* **75** (1978) 2353–2357.
13. Blackmore P., Beebe S., Danforth D. and Alexander N.: Progesterone and 17-alpha-hydroxyprogesterone. *J. Biol. Chem.* **265** (1990) 1376–1380.
14. Majewska M., Harrison N., Schwartz R., Barker J. and Paul S.: Steroid hormone metabolites are barbiturate-like modulators of the GABA receptor. *Science* **232** (1986) 1004–1007.
15. Harrison N. and Simmonds M.: Modulation of the GABA receptor complex by a steroid anaesthetic. *Brain Res.* **323** (1984) 287–292.
16. Gee K.: Steroid modulation of the GABA/benzodiazepine receptor-linked chloride ionophore. *Molec. Neurobiol.* **2** (1988) 291–317.
17. Simmonds M. *Steroids and Neuronal Activity*. John Wiley and Sons, W. Sussex, U.K. (1990) p. 280.
18. Beach F.: *Hormones and Behavior: a Study of Interrelationships between Endocrine Secretions and Patterns of Overt Response*. Paul B. Hoeber, Inc. (1948).
19. Young W., Goy R. and Phoenix C.: Hormones and sexual behavior. Broad relationships exist between the gonadal hormones and behavior. *Science* **143** (1964) 212–218.
20. Lehrman D.: The reproductive behavior of ring doves. *Scient. Am.* **211** (1964) 48–54.
21. Barraclough C. and Leathem J.: Infertility induced in mice by a single injection of testosterone propionate. *Proc. Soc. Exp. Biol. Med.* **85** (1954) 673–674.
22. Phoenix C., Goy R., Gerall A. and Young W.: Organizing action of prenatally administered testosterone propionate on the tissues mediating mating behavior in the female guinea pig. *Endocrinology* **65** (1959) 369–382.
23. Raisman G. and Field P.: Sexual dimorphism in the preoptic area of the rat. *Science* **173** (1971) 731–733.
24. Nottebohm F. and Arnold A.: Sexual dimorphism in vocal control areas of the song-bird brain. *Science* **194** (1976) 211–212.
25. Gorski R., Gordon J., Shryne J. and Southam A.: Evidence for a morphological sex difference within the medial preoptic area of the rat brain. *Brain Res.* **148** (1978) 333–346.
26. Liao S. and Fang S.: Receptor proteins for androgens and the mode of action of androgens on gene transcription in ventral prostate. *Vit. Horm.* **27** (1969) 17–90.
27. Tomkins G., Gelehrter T., Granner D., Martin D., Samuels H. and Thompson E.: Control of specific gene expression in higher organisms. *Science* **166** (1969) 1474–1480.
28. Eisenfeld A. and Axelrod J.: Effect of steroid hormones, ovariectomy, estrogen pretreatment, sex and immaturity on the distribution of 3H-estradiol. *Endocrinology* **79** (1966) 38–42.
29. Kato J. and Villee C.: Factors affecting uptake of estradiol-6, 7-3H by the hypophysis and hypothalamus. *Endocrinology* **80** (1967) 1133–1138.
30. McEwen B. S. and Pfaff D.: Factors influencing sex hormone uptake by rat brain regions: I. Effects of neonatal treatment, hypophysectomy and competing steroid on estradiol uptake. *Brain Res.* **21** (1970) 1–16.
31. McEwen B. S., Pfaff D. and Zigmond R.: Factors influencing sex hormone uptake by rat brain regions: II. Effects of neonatal treatment on testosterone uptake. *Brain Res.* **21** (1970) 17–28.
32. McEwen B. S., Weiss J. and Schwartz L.: Selective retention of corticosterone by limbic structures in rat brain. *Nature* **220** (1968) 911–912.
33. Zigmond R. and McEwen B. S.: Selective retention of oestradiol by cell nuclei in specific brain regions of the ovariectomized rats. *J. Neurochem.* **17** (1970) 889–899.
34. Grosser B., Stevens W., Bruenger F. and Reed D.: Corticosterone binding in rat brain cytosol. *J. Neurochem.* **18** (1971) 1725–1732.
35. McEwen B. S., Magnus C. and Wallach G.: Soluble corticosterone-binding macromolecules extracted from rat brain. *Endocrinology* **90** (1972) 217–226.
36. Knizley H., Jr.: The hippocampus and septal area as primary target sites for corticosterone. *J. Neurochem.* **19** (1972) 2737–2745.

37. McEwen B. S., Weiss J. and Schwartz L.: Retention of corticosterone by cell nuclei from brain regions of adrenalectomized rats. *Brain Res.* **17** (1970) 471–482.
38. McEwen B. S. and Plapinger L.: Association of corticosterone-1,2,3H with macromolecules extracted from brain cell nuclei. *Nature* **226** (1970) 263–264.
39. Michael R.: Oestrogens in the central nervous system. *Br. Med. Bull.* **21** (1965) 87–90.
40. Stumpf W.: Estradiol concentrating neurons: topography in the hypothalamus by dry-mount autoradiography. *Science* **162** (1968) 1001–1003.
41. Pfaff D.: Autoradiographic localization of radioactivity in rat brain after injection of tritiated sex hormones. *Science* **161** (1968) 1355–1356.
42. Attramadal A.: Cellular localization of 3H oestradiol in the hypothalamus: an autoradiographic study in male and female rats. *Z. Zellforsch.* **104** (1970) 572–581.
43. Warembourg M. L.: Fixation de l'oestradiol 3H dans le telencephale et le diencephale chez la souris femelle. *C.R. Soc. Biol.* **164** (1970) 126–129.
44. Sar M. and Stumpf W.: Distribution of androgen target cells in rat forebrain and pituitary after 3H-dihydrotestosterone administration. *J. Steroid Biochem.* **8** (1977) 1131–1135.
45. Sar M. and Stampf W.: Neurons of the hypothalamus concentrate 3H progesterone or its metabolites. *Science* **182** (1973) 1266–1267.
46. Waremborg M.: Radioautographic study of the rat brain, uterus and vagina after 3H R-5020 injection. *Molec. Cell. Endocr.* **12** (1978) 67–69.
47. Gerlach J. and McEwen B. S.: Rat brain binds adrenal steroid hormone: radioautography of hippocampus with corticosterone. *Science* **175** (1972) 1133–1136.
48. Stumpf W.: Autoradiographic techniques and the localization of estrogen, androgen and glucocorticoid in the pituitary and brain. *Am. Zool.* **11** (1971) 725–739.
49. Birmingham M., Stumpf W. and Sar M.: Nuclear localization of aldosterone in rat brain cells assessed by autoradiography. *Experientia* **35** (1979) 1240–1241.
50. Ermisch A. and Ruchle H.: Autoradiographic demonstration of aldosterone concentrating neuron populations in rat brain. *Brain Res.* **147** (1978) 154–158.
51. Stumpf W., Sar M. and Clark S.: Brain target sites for 1,25-dihydroxyvitamin D3. *Science* **215** (1982) 1403–1405.
52. Dratman M., Futaesaku Y., Crutchfield F., Berman N., Payne B., Sar M. and Stumpf W.: Iodine-125-labeled triiodothyronine in rat brain: evidence for localization in discrete neural systems. *Science* **215** (1982) 309–312.
53. Cintra A., Fuxe K., Harfstrand A., Agnati L., Miller L., Greene J. and Gustafsson J-A.: On the cellular localization and distribution of estrogen receptors in the rat tel- and diencephalon using monoclonal antibodies to human estrogen receptor. *Neurochem. Int.* **8** (1986) 585–589.
54. Blaustein J. and Turcotte J.: Estrogen receptor-immunostaining of neuronal cytoplasmic processes as well as cell nuclei in guinea pig brain. *Brain Res.* **495** (1989) 75–82.
55. Blaustein J., King J., Toft D. and TGurcotte J.: Immunocytochemical localization of estrogen-induced progestin receptors in guinea pig brain. *Brain Res.* **474** (1988) 1–15.
56. Warembourg M., Jolivet A. and Milgrom E.: Immunohistochemical evidence of the presence of estrogen and progesterone receptors in the same neurons of the guinea pig hypothalamus and preoptic area. *Brain Res.* **480** (1989) 1–15.
57. Fuxe K., Wikstrom A.-C., Okret S., Agnati L., Harfstrand A., Yu Z.-Y., Granholm L., Zoli M., Vale W. and Gustafsson J.-A.: Mapping of glucocorticoid receptor immunoreactive neurons in the rat tel- and diencephalon using a monoclonal antibody against rat liver glucocorticoid receptor. *Endocrinology* **117** (1985) 1803–1812.
58. Simerly R.: Hormonal control of neuropeptide gene expression in sexually dimorphic olfactory pathways. *TINS* **13** (1990) 104–110.
59. Aronsson M., Fuxe K., Dong Y., Agnati L., Okret S. and Gustafsson J.-A.: Localization of glucocorticoid receptor mRNA in the male rat brain by *in situ* hybridization. *Proc. Natn. Acad. Sci. U.S.A.* **85** (1988) 9331–9335.
60. Herman J., Patel P., Akil H. and Watson S.: Localization and regulation of glucocorticoid and mineralocorticoid receptor messenger RNAs in the hippocampal formation of the rat. *Molec. Endocr.* **3** (1989) 1886–1894.
61. Arizza J., Simerly R., Swanson L. and Evans R. The neuronal mineralocorticoid receptor as a mediator of glucocorticoid response. *Neuron* **1** (1988) 887–900.
62. Bradley D., Young S. and Weinberger C.: Differential expression of alpha and beta thyroid hormone receptor genes in rat brain and pituitary. *Proc. Natn. Acad. Sci. U.S.A.* **86** (1989) 7250–7254.
63. Olton D., Wible C. and Shapiro M.: Mnemonic theories of hippocampal function. *Behav. Neurosci.* **100** (1986) 852–855.
64. Gerlach J., McEwen B. S., Pfaff D., Mokovitz S., Ferin M., Carmel P. and Zimmerman E.: Cells in regions of rhesus monkey brain and pituitary retain radioactive estradiol, corticosterone and cortisol differentially. *Brain Res.* **103** (1976) 603–612.
65. McEwen B. S., Brinton R., Chao H., Coirini H., Gannon M., Gould E., O'Callaghan J., Spencer R., Randall S. and Woolley C.: The hippocampus: a site for modulatory interactions between steroid hormones, neurotransmitters and neuropeptides. In *Neuroendocrine Perspectives* (Edited by E. Muller and R. MacLeod) Springer Verlag, New York (1990) pp. 93–131.
66. McEwen B. S. and Gould E.: Adrenal steroid influences on the survival of hippocampal neurons. *Biochem. Pharmac.* **40,** (1990) 2393–2402.
67. Aus der Muhlen K. and Ockenfels H.: Morphologische veranderungen im diencephalon und telecephalon: storungen des regelkreises adenohypophysenenbennierenrinde. *Z. Zellforsch. Mikrosck. Anat.* **93** (1969) 126–141.
68. Sapolsky R., Krey L. and McEwen B. S.: Prolonged glucocorticoid exposure reduces hippocampal neuron number: implications for aging. *J. Neurosci.* **5** (1985) 1222–1227.
69. Landfield P., Basking R. and Pitter T.: Brain-aging correlates; retardation by hormonal–pharmacological treatments. *Science* **214** (1981) 581–584.
70. Woolley C., Gould E. and McEwen B. S. Exposure to excess glucocorticoids alters dendritic morphology of adult hippocampal pyramidal neurons. *Brain Res.* (1991) In press.
71. Gould E., Woolley E. and McEwen B. S.: Short-term glucocorticoid manipulations effect neuronal morphology and survival in the adult hippocampal formation. *Neuroscience* (1991) In press.
72. Sloviter R., Valiquette G., Abrams G., Ronk E., Sollas A., Paul L. and Neubort S.: Selective loss of hippocampal granule cells in the mature rat brain after adrenalectomy. *Science* **243** (1989) 535–538.
73. Sloviter R.: "Epileptic" brain damage in rats induced by sustained electrical stimulation of their perforant path. I. Acute electrophysiological and light microscopic studies. *Brain Res. Bull.* **10** (1983) 675–697.

74. Nadler J. V. and Cuthbertson G.: Kainic acid neurotoxicity toward hippocampal formation: dependence on specific excitatory pathways. *Brain Res.* **195** (1980) 47–56.
75. Sapolsky R.: Glucocorticoid hippocampal damage and the glutamatergic synapse. *Prog. Brain Res.* (1991) In press.
76. Uno H., Ross T., Else J., Suleman M. and Sapolsky R.: Hippocampal damage associated with prolonged and fatal stress in primates. *J. Neurosci.* **9** (1989) 1705–1711.
77. Gould E., Woolley C., Frankfurt M. and McEwen B. S.: Gonadal steroids regulate dendritic spine density in hippocampal pyramidal cells in adulthood. *J. Neurosci.* **10** (1990) 1286–1291.
78. Woolley C., Gould E., Frankfurt M. and McEwen B. S.: Naturally occurring fluctuation in dendritic spine density on adult hippocampal pyramidal neurons. *J. Neurosci.* **10** (1990) 4035–4039.
79. Loy R., Gerlach J. and McEwen B. S.: Autoradiographic localization of estradiol-binding neurons in rat hippocampal formation and entorhinal cortex. *Dev. Brain Res.* **39** (1988) 245–251.
80. Parsons B., Rainbow T., MacLusky N. and McEwen B. S.: Progestin receptor levels in rat hypothalamic and limbic nuclei. *J. Neurosci.* **2** (1988) 1446–1452.
81. Schumacher M., Coirini H., Frankfurt M. and McEwen B. S.: Regulation of high-affinity GABAa receptors in the dorsal hippocampus by estradiol and progesterone. *Brain Res.* **487** (1989) 178–183.
82. Luine V., Park D., Joh R., Reis D. and McEwen B. S. Estradiol increases choline acetyltransferase activity in specific basal forebrain nuclei and projection areas of female rats. *Expl Neurol.* **89** (1985) 484–490.
83. Luine V. and McEwen B. S.: Sex differences in cholinergic enzymes of diagonal band nuclei in the rat preoptic area. *Neuroendocrinology* **36** (1983) 475–482.
84. Luine V., Park R., Joh R., Reis D. and McEwen B. S.: Immunochemical demonstration of increased choline acetyltransferase concentration in rat preoptic area after estradiol administration. *Brain Res.* **191** (1980) 273–277.
85. Gould E., Frankfurt M., Westlind-Danielsson A. and McEwen B. S.: Sex differences and thyroid hormone sensitivity of hippocampal pyramidal cells. *J. Neurosci.* **10** (1990) 996–1003.
86. Gould E., Allan M. and McEwen B. S.: Dendritic spine density of adult hippocampal pyramidal cells is sensitive to thyroid hormone. *Brain Res.* **525** (1990) 327–329.
87. Westlind-Danielsson A., Gould E. and McEwen B. S.: Thyroid hormone causes sexually distinct neurochemical and morphological alterations in rat septal-diagonal band neurons. *J. Neurochem.* **56** (1991) 119–128.
88. Gould E., Frankfurt M., Westlind-Danielsson A. and McEwen B. S.: Developing forebrain astrocytes are sensitive to thyroid hormone. *Glia* **3** (1990) 283–292.
89. Pavlides C., Westlind-Danielsson A. and McEwen B. S.: Neonatal thyroid hormone treatment attenuates the induction of long-term potentiation in rat hippocampus. *Abstr. Soc. Neurosci.* **2** (1989) 390.17.
90. McEwen B. S., Davis P., Parsons B. and Pfaff D. W.: The brain as target for steroid hormone action. *A. Rev. Neurosci.* **2** (1979) 65–112.
91. Mendelson S. D. and Gorzalka B.: S-HT_1A receptors: differential involvement in female and male sexual behavior in the rat. *Physiol. Behav.* **37** (1986) 345–351.
92. Mendelson S. and Gorzalka B.: Sex differences in the effects of 1-(*m*-Trifluoromethylphynyl) piperazine and 1-(m-Chlorophenyl) piperazine on copulatory behavior in the rat. *Neuropharmacology* **29** (1990) 783–786.
93. Mendelson S. and McEwen B. S.: Chronic testosterone propionate treatment decreases the concentration of [^{3}H]quipazine binding at 5-HT_3 receptors in the amygdala of the castrated male rat. *Brain Res.* (1991) In press.
94. Mendelson S. and McEwen B. S.: Testosterone increases the concentration of [^{3}H]8-hydroxy-2-(di-n-propylamino) tetralin binding at 5-HT_1A receptors in the medial preoptic nucleus of the castrated male rat. *Eur. J. Pharmac.* **181** (1990) 329–331.
95. Segarra A., Mendelson S., Strand F. and McEwen B. S.: Decreased sexual behavior induced by prenatal ACTH is correlated with increased 5-HT1B receptors in the MPN of male rats. *Abstr. ISPNE* **152** (1990).
96. Fischette C., Biegon A. and McEwen B. S.: Sex differences in serotonin 1 binding in rat brain. *Science* **222** (1983) 333–335.
97. Pfaff D.: *Estrogens and Brain Function.* Springer-Verlag, New York (1980).
98. McEwen B. S., Jones K. and Pfaff D.: Hormonal control of sexual behavior in the female rat molecular, cellular and neurochemical studies. *Biol. Reprod.* **36** (1987) 37–45.
99. McEwen B. S., David P., Gerlach J., Krey L., MacLusky N., McGinnis M., Parsons B. and Rainbow T.: Progestin receptors in the brain and pituitary gland. In *Progesterone and Progestin* (Edited by C. Bardin, P. Mauvais-Jarvis and E. Milgrom). Raven Press, New York (1983) pp. 59–76.
100. Schumacher M., Coirini H., Frankfurt M. and McEwen B. S.: Localized actions of progesterone in hypothalamus involved oxytocin. *Proc. Natn. Acad. Sci. U.S.A.* **86** (1989) 6798–6801.
101. Frankfurt M., Gould C., Woolley C. and McEwen B. S.: Gonadal steroids modify dendritic spine density in ventromedial hypothalamic neurons: a golgi study in the adult rat. *Neuroendocrinology* **51** (1990) 530–535.
102. Carrer H. and Aoki A.: Ultrastructural changes in the hypothalamic ventromedial nucleus of ovariectomized rats after estrogen treatment. *Brain Res.* **240** (1982) 221–233.
103. Nottebohm F.: A brain for all seasons: cyclical anatomical changes in song control nuclei of the canary brain. *Science* **214** (1981) 1368–1370.
104. McEwen B. S., Biegon A., Davis P., Krey L., Luine V., McGinnis M., Paden C., Parsons B. and Rainbow T.: Steroid hormones: humoral signals which alter brain cell properties and functions. *Rec. Prog. Horm. Res.* Academic Press, NY **38** (1982) 41–92.
105. Rubin B. and Barfield R.: Progesterone in the ventromedial hypothalamus facilitates estrous behavior in ovariectomized estrogen-primed rats. *Endocrinology* **113** (1983) 979–804.
106. Schumacher M., Coirini H., Pfaff D. and McEwen B. S.: Behavioral effects of progesterone associated with rapid modulation of oxytocin receptors. *Science* **250** (1990) 691–694.
107. Ramirez V. and Dluzen E.: Is progesterone a prehormone in the CNS? *J. Steroid Biochem.* **27** (1987) 1–3.
108. Rainbow T., McGinnis M., Davis P. and McEwen B. S.: Application of anisomycin to the lateral ventromedial nucleus of the hypothalamus inhibits the activation of sexual behavior by estradiol and progesterone. *Brain Res.* **233** (1982) 417–423.
109. Romano G., Mobbs C., Howells R. and Pfaff D.: Estrogen regulation of proenkephalin gene expression in the ventromedial hypothalamus of the rat: temporal qualities and synergism with progesterone. *Molec. Brain Res.* **5** (1989) 51–58.

110. Luine V. and Hearns M.: Relationship of gonadal hormone administration, sex reproductive status and age to monoamine oxidase activity within the hypothalamus. *J. Neuroendocr.* **2** (1990) 1–6.
111. Rainbow T., DeGroff V., Luine V. and McEwen B. S.: Estradiol 17-beta increases the number of muscarinic receptors in hypothalamic nuclei. *Brain Res.* **198** (1980) 239–243.
112. Dohanich G., Witcher J., Weaver D. and Clemens L.: Alteration of muscarinic binding in specific brain areas following estrogen treatment. *Brain Res.* **241** (1982) 347–350.
113. O'Connor L., Nock B. and McEwen B. S.: Regional specificity of gamma-aminobutyric acid receptor regulation by estradiol. *Neuroendocrinology* **47** (1988) 473–481.
114. Schumacher M., Coirini H. and McEwen B. S.: Regulation of high-affinity GABAa receptors in specific brain regions by ovarian hormones. *Neuroendocrinology* **50** (1989) 315–320.
115. Matsumoto A. and Arai Y.: Male-female difference in synaptic organization of the ventromedial nucleus of the hypothalamus in the rat. *Neuroendocrinology* **42** (1986) 232–236.
116. Frankfurt M.: 5,7-dihydroxytryptamine and castration increase dendritic spine density on ventromedial hypothalamic neurons.
117. Segarra A. and McEwen, B. S.: Estrogen increases spine density in ventromedial hypothalamic neurons of peripubertal rats. *Neuroendocr.* (1991) In press.
118. Rainbow T., Snyder L., Berck D. and McEwen B. S.: Correlation of muscarinic receptor induction in the ventromedial hypothalamic nucleus with the activation of feminine sexual behavior by estradiol. *Neuroendocrinology* **39** (1984) 476–480.
119. Rainbow T., Parsons B. and McEwen B. S.: Sex differences in rat brain oestrogen and progestin receptors. *Nature* **300** (1982) 648–649.
120. Brown T., Clark A. and MacLusky N.: Regional sex differences in progestin receptor induction in the rat hypothalamus: effects of various doses of estradiol benzoate. *J. Neurosci.* **7** (1987) 2529–2536.
121. Coirini H. and McEwen B. S.: Progestin receptor induction and sexual behavior by estradiol treatment in male and female rats. *J. Neuroendocr.* **2** (1990) 467–472.
122. Parsons B., Rainbow T., Snyder L. and McEwen B. S.: Progesterone-like effects of estradiol on reproductive behavior and hypothalamic progestin receptors in the female rat. *Neuroendocrinology* **39** (1984) 25–30.
123. Coirini H., Johnson A. and McEwen B. S.: Estradiol modulation of oxytocin binding in the ventromedial hypothalamic nucleus of male and female rats. *Neuroendocrinology* **50** (1989) 193–198.
124. Coirini H., Johnson A., Schumacher M. and McEwen B. S.: Sex differences in the regulation of oxytocin receptor binding by ovarian steroids in the ventromedial hypothalamic nucleus of the rat. *Abstr. Soc. Neurosci.* **16** (1990).
125. O'Keefe J. and Handa R.: Transient elevation of estrogen receptors in the neonatal rat hippocampus. *Abstr. Soc. Neurosci.* **15** (1989) 39.14.
126. Paxinos G. and Watson C. *The Rat Brain in Stereotaxic Coordinates.* Academic Press, N.Y. (1986).