Biomedical Reviews 1997; 7: 51-66
ISSN 1310-392X

STEROID HORMONE RECEPTORS IN THE DEVELOPING BRAIN

Robert J. Handa, Richard H. Price Jr, and Melinda E. Wilson
Department of Cell Biology, Neurobiology and Anatomy, Stritch School of Medicine, Loyola University Chicago, Maywood, Illinois, USA

SUMMARY

• *Sex differences exist in the behavioral patterns of adult vertebrate species. Many of these behavioral differences arise as a, result of the developmental imprinting of sex specific patterns in the anatomical, biochemical and molecular substrates of the brain. It is well established that steroid hormones play an important role during critical periods of development to organize the brain to a male or female specific pattern. In order to demonstrate that these hormones can act directly on brain tissue, ligand specific receptors for estrogen and androgen have been demonstrated in the developing brain, using a variety of techniques. Each class of steroid hormone receptor exhibits a unique distribution in the brain. Furthermore, each receptor undergoes a unique and tissue specific ontogenetic pattern of expression. A careful examination of the distribution and ontogeny of expression allows one to formulate specific hypotheses regarding the role of each of these receptors in the development and maturation of the brain. (Biomed Rev 1997; 7: 51-66)*

Received for publication 5 April 1997 and accepted 25 June 1997.
Correspondence and reprint requests to Dr Robert J. Handa, Department Cell Biology, Neurobiology and Anatomy, Loyola University Medical Center, 2160 South First Ave., Maywood, 11 60153, USA. Tel: 1 (708) 216 3349, Fax: 1 (708)216391, E-mail: rhanda@wpo.it.luc.edu

INTRODUCTION

• It is known that gonadal steroid hormones can act during development to organize the brain in a male or female direction (reviewed in 1). The consequences of gonadal steroid hormone induced brain differentiation ultimately affect adult physiology including many aspects of reproductive behavior (2), neuroendocrine function (2), cognitive function (3-5) and other (6, 7). To determine the mechanisms by which estrogen and androgen can influence the development of the brain, studies have examined the receptors for gonadal steroid hormones in the brains of adult and neonatal animals. These studies have led to the generation of multiple hypotheses regarding the role played by these-hormones in the Cellular processes of sexual differentiation of the brain. Underlying these hypotheses are the observations that steroid hormone receptors are present in sexually differentiated brain areas during the period of time which the tissue is undergoing organization and maturation. This survey briefly reviews the localization, ontogeny and regulation of estrogen receptors (ER) and androgen receptors (AR) in the developing brain.

• Mechanisms of steroid hormone action

The ER and AR belong to the superfamily of ligand-activated nuclear transcription factors which include receptors for other steroid hormones such as glucocorticoids and mineralocorticoids, as well as vitamin D, retinoic acid, thyroid hormone, and a variety of intracellular factors (reviewed in 8-10). Steroid hor-

mone receptors are soluble, high molecular weight intracellular proteins which bind their ligands, 17-J3 estradiol for estrogen receptor (ER), testosterone or dihydrotestosterone for androgen receptor (AR), with high affinity, Kd approximately 1-2x10" $^{1(1}$ M. In the presence of ligand, the receptor is rapidly transformed from an inactive to an active state (9). Transformation results in the transcriptional regulation of steroid hormone responsive genes (10).

It is now well established that the untransibrmed steroid receptor exists as a multiprotein complex associated with heat shock protein (hsp). It is thought that unbound receptors are unable to bind DNA due to the masking of a DNA-binding domain in the receptor by hsp90 (11). Following ligand binding, hsp90 is dissociated from the complex leaving an active form with a high affinity for DNA (12). Untransibrmed receptor is also thought to complex with a variety of other receptor-associated proteins including hsp70 and hsp56 (13). The function of these additional proteins is not yet known, however some, such as the recently described ER-associated protein (ERAP) (14), and an androgen receptor-associated protein (ARAP) may function to modulate the receptor's ability to induce transcription (15).

Ligand binding to the receptor also induces the formation of receptor dimers which are important for recognition and tight binding of the receptor to the response element on DNA (16). The response elements on DNA are comprised of inverted repeals with the two half sites separated by a three nucleotide gap. The palindromic sequence of the DNA response element and the two fold rotational symmetry suggest that the nuclear receptors bind DNA as dimer (17, 18). DNA-binding specificity lor the nuclear hormone receptors is determined, in part, by three residues in the first finger of a zinc region in the DNA-binding domain of the receptor (19).

Target gene specificity may also be conferred by variations between different hormone response elements on DNA, by cell- or tissue-specific regulation of receptor levels, by additional transcription factors which may be necessary for full receptor activity (20), or by tissue-specific hormone metabolism (21). Additional factors in forming a "complex response element" may be involved in directing target gene specificity and in dictating whether the receptor upregulatcs or downregulates transcription. Examples of these have been demonstrated for glucocorticoid, mineraloeorticoid, estrogen and androgen responsive promoter (22-26).

ESTROGEN RECEPTORS

• Typical of all member of the steroid hormone receptor superfamily, the ER is composed of six distinct cassettes or functional domains (Fig. 1; 27-29). The amino terminus (A/B) is hypervariablc and is probably important for regulation and specificity of transcription by theER. Antibodies against ER are generally directed against this region. Region C is the DNA-binding domain and is important in determining target gene specificity (27). The general structure of this region is highly conserved among member of the steroid hormone superfamily and suggests that it performs functions common to all of the receptors (30). Region D is a poorly conserved, hydrophilic domain which can be altered by amino acid deletions without affecting ER function (29, 31). The steroid-binding domain (region E) is believed to bind the ligand by forming a hydrophobic pocket (27). Region E also contains a region for dimerization of the receptor (32) and for ligand-dependent transcriptional activation (33,34). The poorly conserved C-terminal region (F) can be deleted without affecting hormone binding or transcriptional activation (29).

A single form of ER was originally cloned and described for multiple species including the human (27) and rat (28). Recent studies have shown that, in fact, two forms of ER exist. Thus, the originally cloned receptor has been designated ERa, whereas the newly cloned receptor has been designated ERp (35, 36). Both forms of ER exist in the brain (37, 38) possess a high affinity for estradiol and maintain a common domain structure characteristic of steroid hormone receptors. Consequently, although the original studies examining ER binding probably detected both receptor forms, studies using antibodies generated against ERp or probes hybridizing to $mRNA^{HR(i}$ may not necessarily include the P form of receptor. This may help explain some of the reported inconsistencies in the distribution of ER using binding or *in situ* hybridization techniques (37, 39).

$mRNA^{HR}$ isoforms have also been described. An isoform of $mRNA^{HKa}$ lacking exon four, encoding a portion of the ligand-binding domain, has been demonstrated in the hypothalamus (40). Similarly, a smaller form of $mRNA^{KRa}$ has been shown by Northern blot analysis in anterior pituitary (41, 42). Recently, it has been shown that $mRNA^{KRr}$* possesses multiple 5'-untranslated regions (5'-UTR), which are variably spliced onto the first coding exon of the mRNA. These are found at high levels in brain but each with region selectivity. This suggests a differential regulation of the translation of $mRNA^{KRcc}$ in various brain areas (43). The functional significance of these ERa isoforms remains to be determined. The possibility exists that changes in these $mRNA^{HR(I}$ isoforms may occur during development and differentially regulate the sensitivity of developing brain regions to estrogen.

- **Distribution of estrogen receptors in the brain**

ER were initially localized in the adult CNS by *in vivo* autoradiography (44-46). This anatomical distribution coincides with the regions known to mediate many gonadal steroid dependent reproductive behaviors and functions (47,48). Similar anatomical

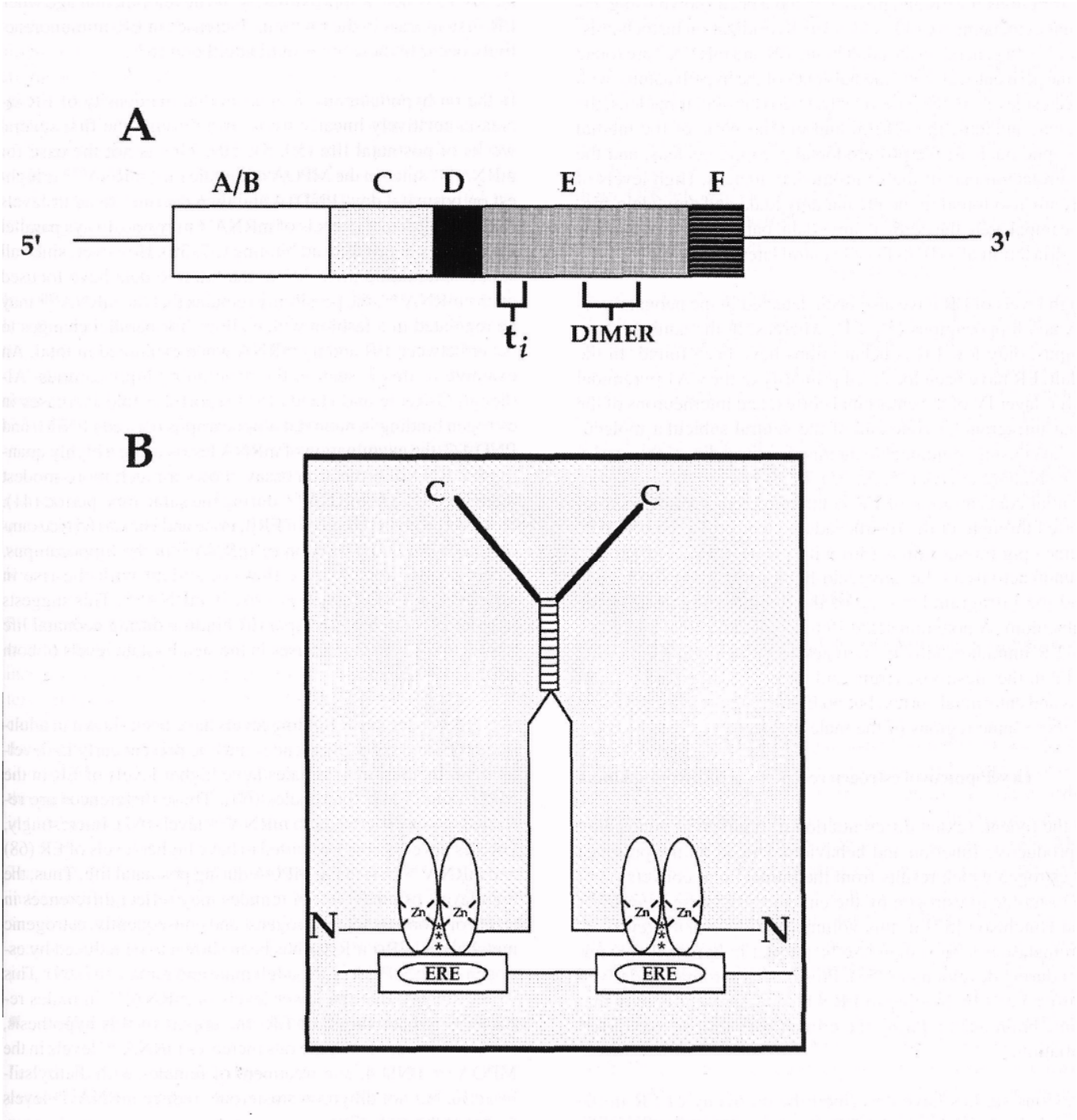

Figure 1. Schematic diagram showing the domain structure of the estrogen receptor (ER) (A). The box represents the protein coding region. Region A/B is the hypervariable, amino terminal domain; C is the DNA-binding domain; D is the hydrophilic domain; E is the ligand-binding domain which contains subdomains for hormone-relieved trancriptional inactivation (Ti) and receptor dimerization (dimer); F is the poorly conserved carboxy terminal domain. The single line represents the 5'- and 3'-untranslated regions of the mRNA^{ER}. A dimer of two estrogen receptors each bound to an estrogen response element (ERE) half site (B). The DNA-binding domain of each ER is represented by the two zinc finger motifs (Zn). C, carboxy terminus; N, amino terminus.

distributions for ER and mRNA^{hlia} have been shown using immunoeytochemistry (49) and *in situ* hybridization histochemistry (37). In general, in the adult brain. ER and mRNA^{KR} are found in the periventricular and medial zones of the hypothalamus, with greatest levels in the anteroventral periventricular nucleus, the arcuate nucleus, the central and medial parts of the medial preoptic nucleus, the posterodorsal preoptic nucleus, and the ventrolateral part of the ventromedial nucleus. High levels of ER arc also found in the medial amygdala and amygdalo-hippocampal area, the cortical amygdala, parts of the bed nucleus of stria terminalis (BNST) and ventral lateral septum.

High levels of ER have also been detected in the neonatal cortex and hippocampus (50, 51), whereas, in the adult hippocampus, only low ER concentrations have been found. In the adult, ER have been localized primarily to the CA1 pyramidal layer, layer IV of the entorhinal cortex, and interneurons of the hilar infragranular zone and of the ventral subicular molecular layer using immunocytochemistry, autoradiography, *and in vitro* binding assays (45, 52-55). In contrast to the rat, a substantial concentration of ER-eontaining neurons has been reported throughout the rostrocaudal extent of the adult female guinea pig hippocampus (56) with greatest levels of ER-immunoreactivily in the amygdalo-hippocampal transition zone and the infragranular zone of the dentate gyrus and in the subiculum. A potential sex difference in entorhinal and cortical ER-immunorcactivity is suggested by the finding of labeled cells in the diestrous, virgin and pregnant mouse hippocampus and entorhinal cortex, but no ER-immunoreactivity is seen in these same regions of the male mouse (49).

- **Development of estrogen receptors in the hypothalamus**

In the rodent, sexual differentiation of brain areas controlling reproductive function and behaviors depend on the presence of estrogen which results from the intracellular conversion of testosterone to estrogen by the enzyme aromatase (see Beyer and Hutchison [57] in this volume of' *Biomedical Reviews).* Aromalase has been shown to be present in hypothalamic nuclei during development (58). Presumably, aromatase derived eslrogen acts by binding to ER which are present in hypothalamic brain nuclei during the critical period of sexual differentiation.

Previous studies have described the ontogeny of ER in the hypothalamus of the rat (50,59), monkey (60), and mouse (61). In general, ER are first found in rat hypothalamus during prenatal development. ER have been detected by at least embryonic day 17-20 (59, 62). In the opossum, a marsupial which is born in a sexually undifferentiated state, ER-immunoreactivity has been shown in the peri ventricular hypothalamus, medial preoptic area (MPOA), lateral septum, BNST, amygdala, arcuate nucleus and ventromedial nucleus (VMN) of the hypothalamus by postnatal day 15 which is approximately the developmental age when ER first appears in the rat brain. Increases in ER-immunoreactivity occur in these areas until adulthood (63).

In the rat hypothalamus, it appears that the density of ER increases relatively linearly from birth through the first several weeks of postnatal life (50, 51, 59). This is not the case for mRNA^{HRp}, since in the MPOA of female rats, mRNA^{hRa} is highest on postnatal day (PND) 4 and then declines to adult levels (64). Changes in the levels of mRNA^{HR} may not always parallel the changes in protein and binding (37, 39). However, since all studies examining mRNA^{KR} in the brain to date have focused on the mRNA^{hRa}, the possibility remains that the mRNA^{KRt5} may be regulated in a fashion which allows for parallel changes to occur between ER and its mRNA when examined in total. An example of this is seen in the developing hippocampus. Although O'Keefe and Handa (51) report 2-3 fold increases in estrogen binding in neonatal hippocampus between PND 0 and PND 4-7, the examination of mRNA levels using a highly quantitative RNAse protection assay shows a much more modest increase (60%) in mRNA^{KRct} during the same time period (41), However, with the cloning of ERp, Price and Handa (65) reexamine the transient expression of mRNA^{HR} in the hippocampus. Their results demonstrate that coincident with the rise in mRNA^{RRa} at PND 4, there is a rise in mRNA^{RRp}. This suggests that increases in hippocampal ER binding during neonatal life may actually reflect increases in the steady-state levels of both ERa and ERP genes.

Sex differences in ER binding levels have been shown in adulthood (66) and these differences may be present early in development. In adult rats, females have higher levels of ER in the MPOA and VMN than males (66). These differences are reflected by sex differences in mRNA$^{HR[i}$ levels (67). Interestingly, females have also been reported to have higher levels of ER (68) and mRNA^{HRp} (64) in the MPOA during postnatal life. Thus, the high levels of mRNA^{KRa} in females may reflect differences in levels of aromatizable androgens, and consequently, estrogenic metabolites. ERoc mRNA has been shown to be reduced by estrogen in the MPOA of the adult male and female (67, 69). This would suggest that the lower levels of mRNA^{hRa} in males reflect *in vivo* activation of ER. In support of this hypothesis, neonatal castration of male rats increases mRNA^{hRa} levels in the MPOA on PND 4, and treatment of females with diethylstilbesterol, but not dihydrotestosterone, reduce mRNA^{hRa} levels to that of the male (70).

- **Development of estrogen receptors in extrahypothalamic brain areas**

Studies have implicated estrogen in the physiology of extrahypothalamic brain areas of the adult. Evidence that ER and mRNA^{HR} are expressed in the hippocampus and cortex of the

adult rat (37, 52, 54,, 71) and guinea pig (56), suggests *a* direct mode of action by which estrogen can influence cortical and hippocampal function. Interestingly, in almost every case, ER are found in relatively low levels in adult rat hippocampus and cortex. On the other hand, studies have demonstrated that ER arc found at high levels in the cortex (72) and hippocampus (51) of the neonatal rat. The high levels of expression of ER in these (issues is transient and exists only during development. The transient expression of ER in these tissues may be useful for defining a critical period of development where certain tissues arc most sensitive to the actions of estrogen.

In cortical tissues it has been shown that ER is found at high levels during the first 2-3 weeks of postnatal life (50, 51). Furthermore, it has been shown that ER are present in migrating cells during differentiation and the establishment of connections. This suggests that ER may play a role in these processes (72). This finding supplements the previous reports that treatment of cortical organotypic cultures of cingulate/frontal cortex with estrogen enhances neurite outgrowth (73) and increases protein synthesis. In contrast, MacLusky *etal* (50) have questioned whether these cortical ER are functional since they were unable to demonstrate receptor occupation by endogenous levels of estrogen.

Studies in the hippocampus have shown that parallel changes occur with those seen in cortical tissues. However, the transient increase in ER in hippocampus occurs earlier than that of coricx. with peak levels at about PND 4-7 (51; see Fig.2). This increase is accompanied by increases in $mRNA^{HR}$ (41,65). Unlike the distribution of ER in the adult hippocampus, the distribution of $mRNA^{HKa}$ in the neonate appears to be more uniform throughout the pyramidal cell layer, with slightly higher levels in CA3 and lower ones in the dentate gyrus granule cells (41).

Transient increases in ER and $mRNA^{HR}$ have also been shown in the auditory cortex (74) and the facial nucleus (75-77). It is suggested that they may be involved in the maturation of the auditory system since the ER-conlaining cells in the facial nucleus project to the posterior auricular muscles (75).

- **Regulation of estrogen receptors during development**

Few studies have examined the factors which cause ER to be expressed or not expressed in various tissues during development. Using *in vitro* autoradiography, Kuhnemann *et al* (78) have demonstrated that castration of male rats at birth increase ER levels in the preoptic area and ventromedial and arcuate nucleus, whereas injection of female rats at birth with testosterone propionate decreases ER binding. Consistent with these observations, DonCarlos *et al* (70) have demonstrated that $niRNA^{11<p}$ can be autologously regulated by estrogen in the neonate. In these studies, castration of male rats increases $mRNA^{hR\iota 3}$ whereas treatment of female neonates with diethylstibesterol reduces $mRNA^{KRl5}$ to the level of the male.

These studies suggest that autoregulation of transcription is present in the neonate, in a fashion similar to that described in the adult (67, 69, 79, 80). However, little is known about the intracellular factors which regulate developmental increases in ER. Using a heterochronic and heterotopic transplant paradigm, O'Keefe *etal (S I)* and Pederen *et al* (82) have determined that factors driving the transient developmental increases in ER in the hippocampus and cortex are determined early in gestation, and are an inherent developmental program to which the tissue is committed. Similar results have been shown for hypothalamus (81, 83). Transplantation of neonatal hypomalamic tissue to the cortex (81) or the choroidal pia overlying the superior colliculus (83) does not alter the developmental pattern of ER expression. Furthermore, the appearance of ER is not dependent on donor sex or gonadal steroid environment (83). Thus, although these studies do not determine the intracellular factors responsible for developmental increases in ER, they do appear to rule out paracrine and endocrine factors as influencing the autonomous, region specific developmental profile of ER expression.

ANDROGEN RECEPTORS

- **Characterization and distribution of androgen receptors in the brain**

Early studies, using *in vivo* receptor autoradiography, demonstrated specific patterns of radio!abeled hormone uptake by cells in the central nervous system of the adult (84). Androgen concentrating cells were shown to have a relatively wide distribution throughout the brain of many species including the rat (85), guinea pig (86), non-human primate (87) and sheep (88). Most notably, androgen concentrating cells were found at relatively high levels in specific nuclei of the brain including the VMN, arcuate nucleus, periventricular nucleus, MPOA, BNST, hippocampal CA1 pyramidal cells, and medial amygdala. Subsequent studies, using *in vitro* binding assays, showed that following cell fractionation, cytosolic preparations, and nuclear salt extracts of tissue taken from specific brain regions contained a single, saturable receptor for radiolabeled dihydrotestosterone or Rl 881, a synthetic androgen, with a high affinity for ligand (0.2-2 nM) (89, 90). Quantitation of the levels of AR, using *in vitro* binding assays on microdissected brain tissue, revealed a similar brain distribution and relative amount of AR (90) as that revealed by *in vivo* autoradiographic techniques. Finally, the dynamics of the AR were revealed by the use of *in vitro* binding assay. That is, in the absence of ligand, such as following castration of male rats, most receptors are found in the cytosolic or unoccupied form. Following treatment of animals with hormone, the receptors become occupied and are measurable fol-

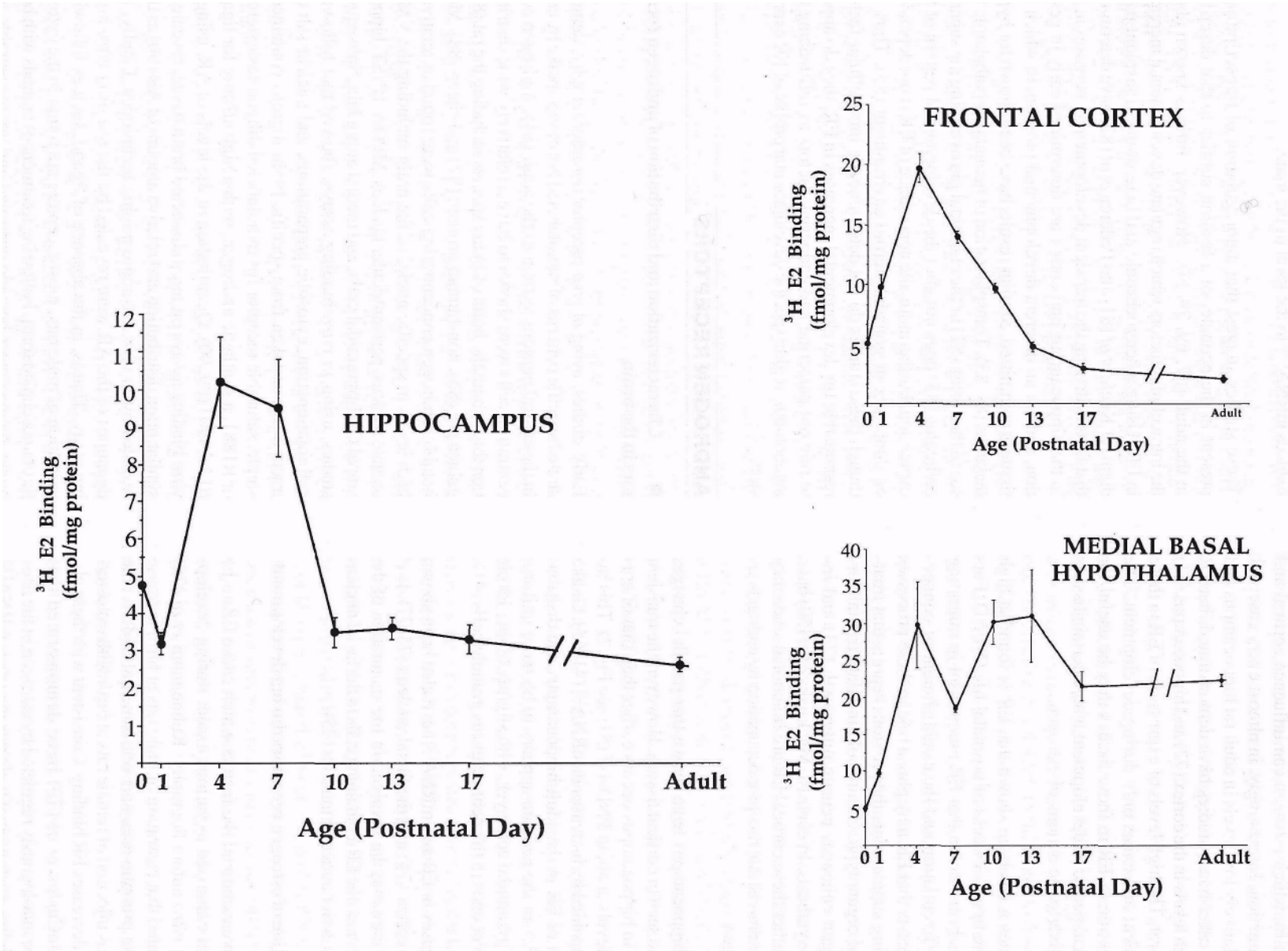

***Figure** 2. Developmental profile of estrogen receptors in the hippocampus, frontal cortex and hypothalamus/preoptic area of the rat Each point represents the mean ± SEM. Postnatal day 0, day of birth. No significant sex differences are detected and thus, data from males and females are combined for this figure. From Ref. 51, with permission*

lowing extraction from nuclei by high salt buffer (91,92).

Recent studies have used immunocytochemical and molecular techniques to accurately map the distribution of the AR and their inRNA in the brain. In most cases, the distribution of AR-immunorcactivity and $mRNA^{AK}$ match that described for AR using autoradiography (37, 93, 69). An example of the distribution of $mRNA^{Ali}$ is shown in Fig.3. In this study, *in situ* hybridization was performed using a probe detecting both known forms of $niRNA^{Ali}$ and thus, represents the distribution of total $mRNA^{AR}$.

Early studies, examining the differential effects of testosterone and dihydrotestosterone on a variety of end points, suggested the possibility that two specific AR existed, one for testosterone and another for dihydrotestosterone (94). It now appears that AR are transcribed from a single gene of approximately 75-90 Kb (95). However, differences in the functional capacity of testosterone and dihydrotestosterone to activate gene expression appear to be related to their relative differences in interacting with a single AR. Dihydrotestosterone possesses a stronger affinity for the AR, stabilizes the receptor to a greater extent than testosterone, and may cause conformational changes in the AR which allows it to interact with the androgen response element on DNA more efficiently (96).

Studies have also shown that the $mRNA^{Al<}$ exists in several forms in the rat brain. Although the predominant form of $mRNA^{AR}$ in peripheral tissues is one of approximately 10-11 Kb, a second smaller form of $mRNA^{AR}$ (approximately 9.0-9.5 Kb) exists in brain at relatively high levels (97, 98). In some tissues, such as the cortex, this smaller form of $mRNA^{Al<}$ predominates (97). Using 5'- RACE to amplify the untranslated region of the small form of $mRNA^{AR}$, Price and Handa (99) demonstrate that the smaller form ol'$mRNA^{Al<}$ is due, in part, to the presence of a truncated 5'-UTR. Studies examining the 5'-UTR of AR have shown that removal of the 5'-UTR results in a decreased translational efficacy (100), thus suggesting that the presence of the smaller form of $mRNA^{AR}$ in hrain tissue may represent an additional mechanism used by neural tissues to regulate AR translation.

- **Ontogeny of androgen receptors in the brain**

A role for AR in the sexual differentiation of the brain has been suggested by studies showing that androgen treatment of gonadcctomized males or females can result in the masculinization or del'eminization of behaviors expressed in adulthood (101). In rats, for example, rough and tumble play is differentiated in the masculine direction by dihydrotestosterone but not estrogen (reviewed in 102). Androgens acting through the AR also appear to be particularly important in the sexual differentiation of the primate brain. In the rodent, some behaviors, such as aggression and infanticide, may be differentiated in the male direction by androgens acting in concert with estrogen (103). These studies suggest that AR should be found in the brain areas which are important for these behaviors during the time period in which they are differentiating.

Several studies have examined the ontogeny of AR in various brain regions. Using *in vitro* binding assays, Vito and Fox (59) and Lieberburg *et al* (104) have demonstrated that AR are present in the developing rat brain as early as embryonic day 20. The concentration of AR in the hypothalamus rises during the first four weeks of life to eventually achieve adult levels. Fig.4 demonstrates a typical developmental profile for AR in the rat hypothalamus-preoptic area in the absence of modulation by circulating steroid. In this study, male and female rats were gonadcctomized on the day of birth (PND 0). AR levels were measured by *in vitro* binding assay using ^{3}H-R188I as the radioligand. Binding was determined at PND 1,12, 20, 30 and adulthood (3 months of age, males only). Gonadectomy was performed in order to reduce circulating hormones which might interfere with the assay and to allow examination of the development of AR in the absence of influence by endogenous gonadal steroid hormones. Significant increases in unoccupied receptor are noted during the first 30 days of life and there were no sex differences. Highest levels were found on PND 30 and slight decreases occurred afterward to adult levels. Thus, these data suggest that the development of AR in the hypothalamus is delayed relative to that of ER.

The development of AR in the brain has also been examined using immunocytochemical techniques (105). Consistent with • binding data, it appears that AR-immunoreactivity increases during postnatal life but initially appears at various times during postnatal development depending on the brain region. Some areas (ventral premammillary nucleus) show staining as early as PND 1 whereas other (posterior dorsal medial amygdala) do not exhibit staining until later in development. Similar to the results of studies using arat model, in the ferret, increases in AR-immunoreactivity also occur during postnatal development (106). Peaks in AR-immunoreactivity occur at the time of puberty in the MPOA, VMN of the hypothalamus, arcuate nucleus, and medial amygdala, but not in the septum orBNST (106). Interestingly, AR-immunoreactivity can be further increased by exposure to long day lengths, a paradigm which hastens the onset of puberty in male ferrets (106).

Recently, molecular techniques have been used to determine if $mRNA^{AR}$ is being expressed during perinatal development. Using Northern blot analysis, $mRNA^{AR}$ has been detected in the hypothalamus as early as PND 7(107, Fig.5). Unfortunately, this study only used probes which detected the larger form of $mRNA^{AR}$, thus, whether the smaller form of $mRNA^{AR}$ is present during development, or the ratio of large to small form of $mRNA^{AR}$ undergoes developmental alterations remains to be determined. In this study, distinct bands at earlier ages were not always con-

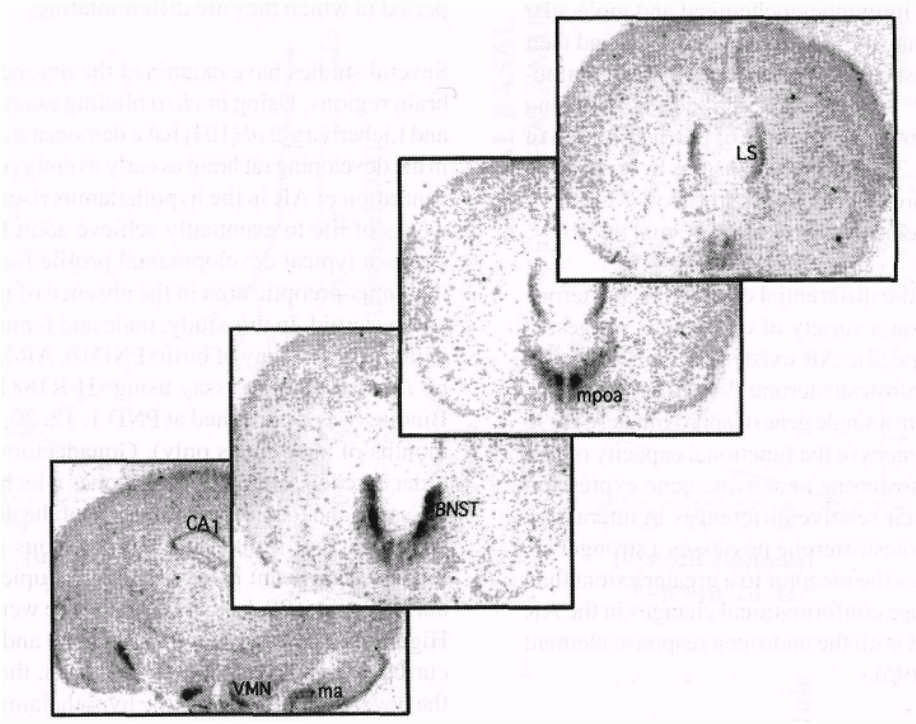

Figure 3. Film auto radio grams showing the distribution ofmRNA^{AR} in the brain of the adult male rat as detected using in situ hybridization analysis. The probe used in this study recognizes both the small and large forms ofmRNA^{AH}. CA1, CA1 pyramidal layer of hippocampus; VMN, ventromedial nucleus; ma, medial amygdala; mpoa, medial preoptic area, BNST, bed nucleus of the stria terminalis; LS, lateral septal nucleus.

sistently found, however, studies using *in situ* hybridization have shown that mRNA^{AK} is present in specific brain nuclei as early as PND 0 (108). Interestingly, McAbeeef *al* (108) also demonstrated a sex difference in mRNA^{AK} in the MPOA and BNST, hut not the VMN, with males having greater levels of receptor mRNA than females. The developmental profile of mRNA^{AR} roughly parallels that of AR binding. The demonstration of sex differences in mRNA^{AK} as early as PND 4 may be the result of regulation of the AR gene by the neonatal steroid hormone milieu. Neonatal castration of males reduces mRNA^{AR} levels measured in PND 10 animals and treatment of neonatally castrated males with androgen increases the levels of mRNA^{AR} (109) in the MPOA and BNSTof the PND 10 rat. How these findings concerning mRNA$^{A"}$ levels relate to AR binding or protein levels, and whether they represent early organizational events in the differentiation of the AR system or transient activational effects of hormone on AR gene expression remains to be determined.

The monkey and guinea pig are two species in which sexual differentiation of the brain occurs prior to parturition and which depend predominantly upon non-aromatizable androgens for brain sexual differentiation (110). In these species, developmental increases in AR occur during gestation. Using *in vitro* binding assay, Handa *et al* (87) demonstrated that AR could be detected as early as day 50 of gestation (the end of the first trimester of pregnancy) in the fetal rhesus monkey. The greatest increases in AR occur throughout primate gestation (approximately 160 days) with high levels of AR found in fetal monkey hypothalamus during late gestation and further, but less substantial increases to adult levels. In the guinea pig, AR binding increases during the last half of gestation (E 30-60), at a time when sexual differentiation of the brain has been occurring (110).

AR have also been identified in areas outside the hypothalamus such as the cortex, amygdala and hippocampus (37, 90). Similarly to that demonstrated for ER, these areas may show developmental changes in receptors which are different from that of the hypothalamic nuclei. In the rhesus monkey fetus, for example, a transient increase in AR ligation was demonstrated in

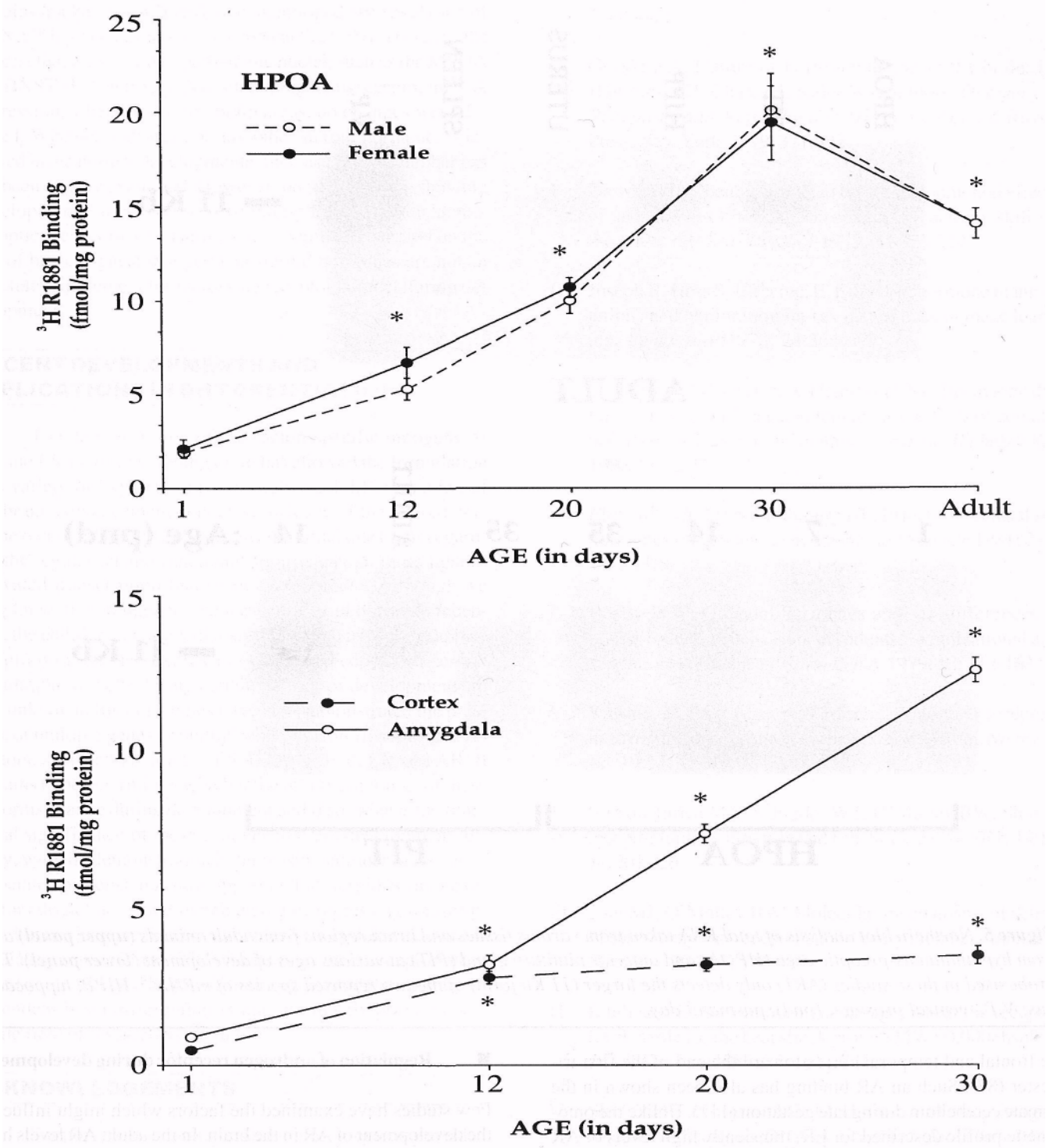

***Figure** 4. Ontogeny ofandrogen receptor in various brain areas as determined using in vitro binding assay. HPOA, hypothalamus-preoptic area. Animals were gonadectomized on postnatal day 0 (day of birth) and sacrificed at the indicated ages. Each point represents the mean .±SEM of 8 - 20 determinations. There are no sex differences detected and thus, in the lower panel, data from males and females are pooled for graphic presentation. * represents those points significantly different ($p<0.05$)from the PND 0 group.*

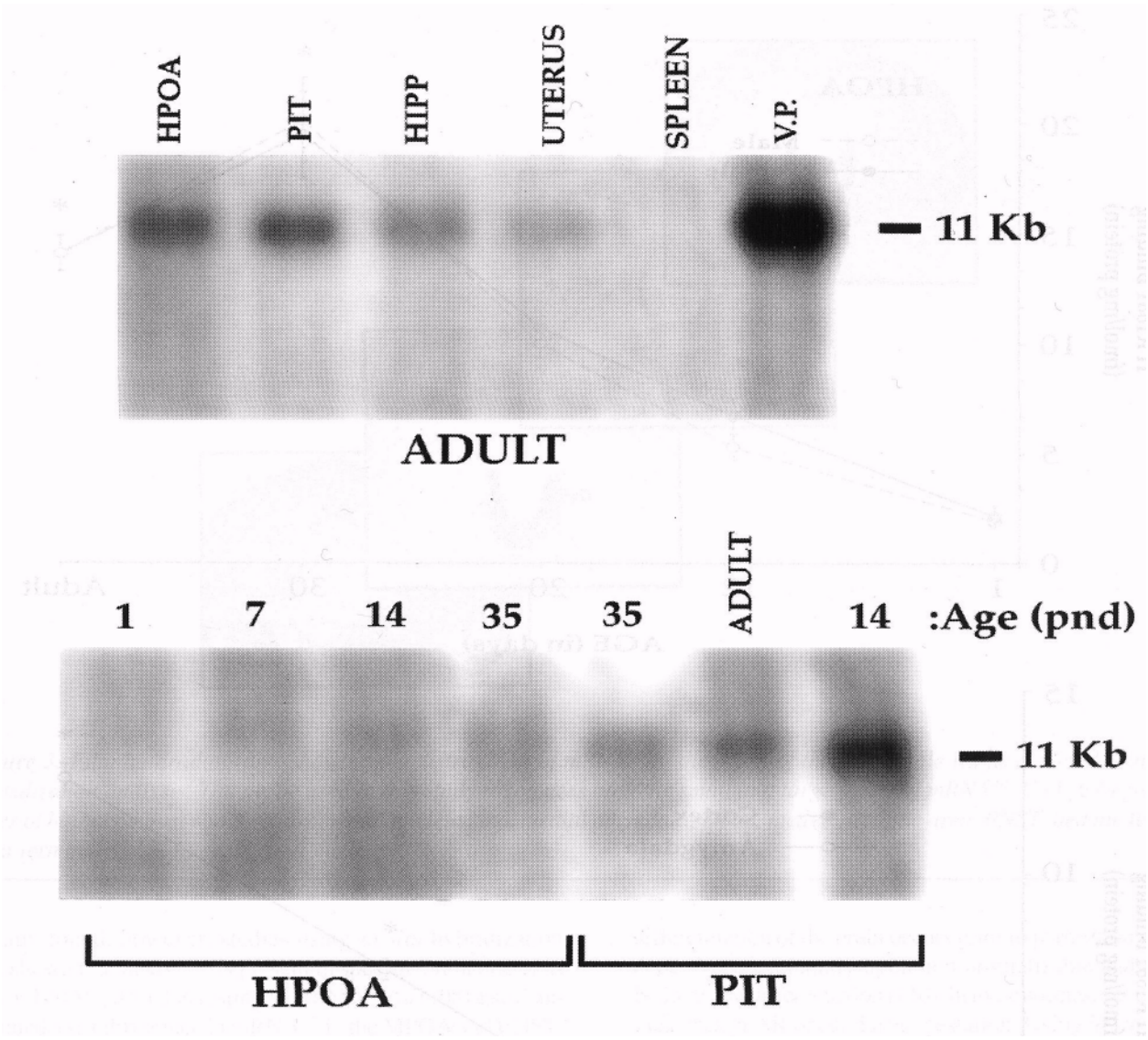

***Figure** 5, Northern blot analysis of total RNA taken from various tissues and brain regions from adult animals **(upper panel)** and from hypot.halamus-preoptic area (HPOA) and anterior pituitary gland (PIT) at various ages of development, **(lover panel).** The probe used in these studies (ART) only detects the larger (11 Kb form) of the two reported species ofmRNA^{AR}. HIPP, hippocampus; V.P., ventral prostate; (pnd), postnatal day.*

the frontal and temporal cortex toward the end of the first trimester (87). Such an AR binding has also been shown in the primate cerebellum during late gestation (111). Unlike the ontogenelic profile described for ER, transiently high levels of AR binding do not occur during the development of the rat cortex (Fig.4). Whether these increases in AR binding reflect an increase in sensitivity of these tissues to androgen remains to be determined. However, the possibility exists that transient changes in receptor number could define a critical period for the organizational actions of andros;en on certain brain structures.

- **Regulation of androgen receptor during development**

Few studies have examined the factors which might influence the development of AR in the brain. In the adult, AR levels have been reported to be upregulated in some brain regions by castration or estrogen treatment (90, 91), and downregulated following progesterone treatment (112). In contrast, mRNA^{AR} has been shown to increase (69, 98, 113), decrease (69, 114), or not change (115), depending on the brain region, time after castration or hormone replacement, or method of detection. Whether

these same regulatory processes occur during development remains not known. As previously mentioned, the regulation of $mRNA^{AR}$ has been examined in.neonatal life (109). These results suggest that for several hypothalamic nuclei, such as theMPOA and BNST, that androgen exposure upregulates receptor mRNA expression, whereas in other brain areas, no changes were detccled. Whether androgen, or any other steroid hormone, is involved in the normal developmental increases in AR number has not been closely examined. However, our data (Fig.4), showing developmental increases in AR binding in the hypothalamusprcoplic area, cortex and amygdala of animals castrated on the day of birth, suggest that gonadal steroid hormones are not an absolute requirement for increasing receptor number during development.

RECENT DEVELOPMENTS AND IMPLICATIONS IN DIFFERENTIATION

• The demonstration of the region-specific ontogeny of AR and ER in the developing brain has allowed the formulation of a variety of hypotheses regarding sexual differentiation of the brain. Advancements in our knowledge of the steroid hormone receptor biology have raised additional questions regarding the regulation and functional significance of these ligand-activated transcription factors in development. Although we now know the ontogenetic patterns of steroid hormone receptors, the cellular and molecular signals which trigger the apparent pre-programmed expression of these receptors in certain populations of cells during certain periods of development are still unknown. Recent studies have also demonstrated the presence of multiple genes or multiple mRNA isoforms for some receptors, such as the variety of 5'-UTR for both ER and AR. It remains lo be determined whether the relative amounts of these isoforms change during development and if so, what is the functional significance of these changes in brain development. Finally, with the demonstration of increasing number of receptor-associated proteins, it is now apparent that steroid hormone receptors do not act alone in enhancing or repressing transcription, but are just one part of a complex intracellular machinery regulating transcription. The ontogeny of these receptor-associated-proteins in the brain and their role in modulating steroid-dependent brain differentiation may ultimately prove to be a fertile field of research in the future.

ACKNOWLEDGEMENTS

• Support for the aulors' research program was provided by grants from the National Science Foundation and National Institutes of Health.

REFERENCES

Arnold AP, Gorski RA. Gonadal steroid induction of structural sex differences in the CNS. *Annu Rev Neurosci* 1984; 7:413-442

2. Gorski RA. Nature of hormone action in the brain. In: Hamilton TH, Clark JH, Sader WA, editors. *Ontogeny of Receptors and Reproductive Hormone Action.* Raven Press, New York, 1979; 371-392

3. Dawson JL, Cheung YM, Lau RT. Developmental effects of neonatal sex hormones on spatial and activity skills in the white rat. *Biol Psychol* 1975; 3: 213-229

4. Joseph R, Hess S, Birecree, E. Effects of hormone manipulations and exploration on sex differences in maze learning. *Behav Biol* 1978; 24:364-377

5. Van Haaren F, Van Hest A, Heinsbroek RP. Behavioral differences between male and female rats: effects of gonadal hormones on learning and *memory. Neurosci Biobehav Rev* 1990; 14:22-33

6. Ehrhardt AA, Meyer-Bahlburg HF. Effects of prenatal sex hormones on gender-related behavior. Science 1981; 211: 1312-1318

7. Beatty WW. Gonadal hormones and sex differences in nonreproductive behaviors in rodents: organizational and activational influences. *Harm Behav* 1979; 12: 112-163

8. Kawata, M. Role of steroid hormones and their receptors in structural organization of the nervous system. *Neurosci Res* 1995; 24:1-46 .

9. Carson-Jurica MA, Schrader WT, O'Malley BW. Steroid receptor family: structure and functions. *EndocrRev* 1990; 11:201-220

10. Tsai MJ, O'Malley BW. Molecular mechanisms of action of steroid/thyroid receptor superfamily member. *Annu Rev Biochem* 1994; 63:451-486

11. Baulieu, EE. Steroid hormone antagonists at the receptor level: a role for the heat shock protein MW 90,000 (hsp90). *J Cell Biochem 1981; 35:* 161-174

12. Skafar DF, Notides AC. Modulation of the estrogen receptor's affinity forDNA by *estradio\JBiol Chem* 1985; 260:12208-12213

13. Sanchez ER. Hsp56: a novel heat shock protein associated with untransformed steroid receptor complexes. *J Biol Chem* 1990;265:22067-22070

14. Halachmi S, Marden E, Martin G, MacKay H, Abbondanza C, Brown M. Estrogen receptor-associated proteins: possible mediators of hormone-induced transcription. *Science* 1994;264: 1455-1458.

15. Yeh S, Chang C. Cloning and characterization of a specific coactivator, ARA_{70}, for the androgen receptor in human prostate cells. *Proc Natl AcadSci USA* 1996; 93:5517-5521

16. Kumar V, Chambon P. The estrogen receptor binds tightly to its responsive element as a ligand-induced homodimer. *Cell* 1988; 55: 145-156

17. Green S, Chambon P. Nuclear receptors enhance our understanding of transcription regulation. *Trends* Gene? 1988; 4:309-313

18. Forman BM, Samuels HH. Dimerization among nuclear hormone receptors. *New Biol* 1990; 2: 587-594

19. Mader S, Kumar V, de Verneuil H, Chambon P. Three amino acids of the oestrogen receptor are essential to its ability to distinguish an oestrogen from a glucocorticoid response element. *Nature* 1989; 33: 271-274

20. Meyer ME, Gronemeyer H, Turcotte B, Bocquel, MT, Tasseet D, Chambon P. Steroid hormone receptors compete for factors that mediate their enhancer function. *Cell* 1989; 57:433-442

21. Funder JW, Pearce PT, Smith R, Smith AI. Mineralocorticoid action: target tissue specificity is enzyme, not receptor mediated. *Science* \988; 242:583-585

22. Diamond MI, Miner JN, Yoshinaga SK, Yamamoto KR. Transcription factor interactions: selectors of positive or negative regulation from a single DNA element. *Science* 1990; 249: 1266-1272

23. McDonnell DP, Shahbaz MM, Vegeto E, Goldman ME. The human progesterone receptor A-form functions as a transcriptional modulator ofmineralocortocoid receptor transcriplional activity.7 *SteroidBiochemMolBiol* 1994; 48: 425-432

24. Tzukerman MT, Esty A, Santiso-Mere D, Danielian P, Parker MG, Stein RB *et al.* Human estrogen receptor transactivational capacity is determined by both cellular and promoler context and mediated by two functionally distinct intramolecular regions. *Mol Endocrinol* 1994; 8:21 -30

25. Webb P, Lopez GN, Uht RM, Kushner PJ. Tamoxifen activation of the estrogen reccptor/AP-1 pathway: potential origin for the cell-specific estrogen-like effects of antiestrogens. *Mol Endocrinol* 1995; 9:443-456

26. Adler AJ, Scheller A, Robins DM. The stringency and magnitude of androgen-specific gene activation are combinatorial functions of receptor and nonreceptor binding site sequences. *Mol Cell Biol* 1993; 13: 6323-6335

27. Green S, Walter P, Kumar V, Krust A, Bornert JM, Argos P *et al.* Human oestrogen receptor cDNA: sequence, expression and homology to v-erb-A.Atowre 1986; 320: 134-139

28. Koike S, Sakai M, Muramatsu M. Molecular cloning and characterization of rat estrogen receptor cDNA. *Nucl AcidsRes* 1987; 15:2499-2513

29. Kumar V, Green S, Staub A, Chambon P. Localization of the oestradiol-binding and putative DNA binding domains of the human oestrogen receptor. *EMBO J* 1986; 5: 2231-2236

30. Evans RM. The steroid and thyroid hormone receptor superfamily. *Science* 1988; 240:889-895

31. Kumar V, Green S, Stack G, Berry M, Jin J, Chambon P. Functional domains of the human estrogen receptor. Ce//1987;51:941-951

32. Fawell SE, Lees JA, White R, Parker MG. Characterization and colocalization of steroid binding and dimerization activities in the mouse estrogen receptor. *Cell* 1990; 60:953-962

33. Webster NJG, Green S, Rui Jin J, Chambon P. The hormone binding domains of the estrogen and glucocorticoid receptors contain an inducible transcription activation function. Ce//1988;54:199-207

34. Lees JA, Fawell SE, Parker MG. Identification of two transactivation domains in the mouse estrogen receptor. *Nucl Acids Res* 1989; 17: 5477-5488

35. Kuiper GG, EnmarkE, Pelto-Huikko M, Nilsson E, Gustafsson JA. Cloning of a novel estrogen receptor expressed in rat prostate and ovary. *Proc NatlAcadSci USA* 1996; 93:5925-5930

36. MosselmanS,PolmanJ,DijkemaR.ERp: identification and characterization of a novel human estrogen receptor. *FEES Lett* 1996; 392:49-53

37. Simerly RB, Chang C, Muramatsu M, Swanson LW. Distribution of androgen and estrogen receptor mRNA-contain-

ing cells in the rat brain: an *in situ* hybridization study. *J Comp Neural* 1990; 294:76-95

38. Shughrue P, Komm B, Mcrchenthaler I. The distribution of cstrogen receplor-(3 mRNA in the rat hypothalamus. *Steroids* 1996; 61: 678-681

39. Toran-Allcrand CD, Miranda RC, Hochberg RB, MacLusky NJ. Cellular variations in estrogen receptor mRNA translation in the developing brain: evidence from combined [^{125}I] estrogen autoradiography and non-isotopic *in situ* hybridization histochemislry.jBraw *Res* 1992; 576:25-41

40. Skipper JK, Young LI, Bergeron JM, Tetzlaff MT, Osborn CT, Crews D. Identification of an isoform of the estrogen receptor messenger RNA lacking exon four and present *mlhebra'm.ProcNatlAcadSciUSA* 1993; 90: 7172-7175

41. O'Kcefe JA, Li Y, Burgess LH, Handa RJ. Estrogen receptor mRNA alterations in the developing rat hippocampus. *MolBrain Res* 1995; 30: 115-124

42. Shupnik MA, Gordon MS, Chin WW. Tissue specific regulation of rat estrogen receptor mRNAs. *Mol Endocrinol* 1989;3:660-665

43. Hirata S, Koh T, Yamakamouri N, Hoshi K, Kato J. The untranslated first exon, "exon OS" of the rat estrogen receptor (ER) gene. *FEES Lett* 1996; 394: 371-373

44. Sheridan PJ. Estrogen binding in the neonatal neocortex. *Brain Res* 1979; 178:201-206

45. PfatTD, Keiner M. Atlas of estradiol-concentrating cells in the central nervous system of the female rat. *J Comp Neural* 1973; 151: 121-158

50. MacLusky NJ. The development of estrogen receptor systems in the rat brain and pituitary: postnatal development. *Brain Res* 1979; 178: 143-160

51. O'Keefe JA, Handa RJ. Transient elevation of estrogen receptors in the neonatal rat hippocampus. *Dev Brain Res* 1990; 57: 119-127

52. Weiland NG, Orikasa M, Hayashi S, McEwen BS. Localization of estrogen receptors in the hippocampus of male and female rats. *Soc Neurosci Abst* 1996; 22: 618

53. Loy R, Gerlach JL, McEwen BS. Autoradiographic localization of estradiol binding neurons in the rat hippocampal formation and entorhinal cortex. *Dev Brain Res* 1988; 39: 245-251

55. Rainbow TC, Parsons B, MacLusky NJ, McEwen BS. Estradiol receptor levels in rat hypothalamic and limbic nuclei. *J Neurosci* 1982; 2: 1439-1445

56. DonCarlos LL, Monroy E, Morrell J. Distribution of estrogen receptor-immunoreative cells in the forebrain of the female guinea pig. *J Comp Neurol* 1991; 305: 591-612.

57. Beyer C, Hutchion JB. Developmental profile and regulation of brain estrogen synthesis by *aromalase.BiomedRev* 1997;7:41-50

58. George FW, Ojeda SR. Changes in aromatase activity in the rat brain during embryonic, neonatal, and infantile *development. Endocrinology* 1982; 111: 522-529

59. Vito CC, Fox TO. Androgen and estrogen receptors in embryonic and neonatal rat brain. *Dev Brain Res* 1982; 2: 97-110

tain ing cstrogen receptor-like immunoreactivity in the Brazilian opossum brain. *Brain Res Dev Brain Res* 1991; 63: 209-219

64. DonCarlos LL, Handa RJ. Developmental profile of estrogcn receptor mRNA in the preoptic area of male and female neonatal rats. *Dev Brain Res* 1994; 79:283

65. Price Jr RH, Handa RJ. Transient expression of estrogen receptor (ER) |3 mRNA in developing rat hippocampus. *Ahst Eudocr Soc* 1997; In press

66. Brown T.I, Hochherg RB, Zielinski JE, MacLusky NJ. Regional sex differences in cell nuclear estrogen binding capacity in the rat hypothalamus and preoptic area. *Endocrinology* 1988; 123: 1761-1770

67. Shughrue PJ, Bushncll CD, Dorsa DM. Estrogen receptor mRNA in female rat brain during the estrous cycle: a comparison with ovariectomized females and intact males. EM-*docrhwlogy* 1992; 131: 381 -388

68. Kuhnemann S, Brown TJ. Hochbcrg, RB, MacLusky NJ. Sex differences in the development of estrogen receptors in the rat brain. Worm *Behav* 1994; 28:483-491

69. Handa RJ, Kerr JE, DonCarlos LL, McGivern RF, Hejna G. Hormonal regulation of androgen receptor messenger RNA in the medial preoptic area of the male rat. *Mol Brain Res* 1996;39:57-67

70. DonCarlos LL, McAbcc M, Ramer-Quinn DS, Stancik M. Estrogen receptor mRNA levels in the preoptic area of neonatal rats arc responsive to hormone manipulation. *Brain Res* Dev *Brain Res* 1995; 84: 253-260

71. Maggi A, Susanna L. Bellini E, Mantero G, Zucchi I. Hippocampus: a target for estrogen action in mammalian brain. *Mol Endocrinol* 1989; 3: 1165-1170

72. Shughrue PJ, Stumpf WE, MacLusky NJ, Zielinski JE, Hochberg RB. Developmental changes in estrogen receptors in mouse cerebral cortex between birth and post weaning: studied by autqradiography with 11 Fj-methoxy-16 (X-iodoestradiol.Ew/ornno/os.v 1990; 126:1112-1124

73. Toran-Ailerand CD. On the genesis of sexual differentiation of the central nervous system: morphogenetic consequences of stcroidal exposure and possible role of oc-fetoprolcin. *Prog Brain Res* 1984; 61: 63-98

74. Yokusaka M, Okamura H, Hayashi S. Transient expression ol'estrogen receptor-like immunoreactivity (ER-LI) in layer V of the developing rat cerebral cortex. Dev *Brain Res* 1995; 84:99-108

75. Yokusaka M, Hayashi S. Transient expression of estrogen receptpr-immunoreactivity (ER-LI) in the facial nucleus of the neonatal rat. *Neurosci Res* 1992; 15: 90-95

76. Hayashi S. Immunocytochemical detection of estrogen receptor in the facial nucleus of the newborn rat by three antibodies with distinct epitopes. /form Bg/zav 1994; 28: 530-536

77. Orikasa C, Okamura H, Hayashi S. Estrogen receptor found in the facial nucleus of the newborn rat is suppressed by exogenous estrogen: immuno- and m j/f» hybridization histochemical studies.Devgmm/?^ 1994; 82:9-17

78. Kuhnemann S, Brown TJ, Hochberg RB, MacLusky NJ. Sexual differentiation of estrogen receptor concentrations in the rat brain: effects of neonatal testosterone exposure, gram/?e; 1995; 691: 229-234

79. Simerly RB, Young BJ. Regulation of estrogen receptor messenger ribonucleic acid in rat hypothalamus by sex steroid hormones. Mo/Endocrmo/1991; 5:424-432

80. Lisciotto CA, Morrell JI. Circulating gonadal steroid hormones regulate ER mRNA in

81. O'Keefe JA, Pederen EB, Castro AJ, Handa RJ. The ontogeny of estrogen receptors in hetcrochronic hippocampal and ncocortical transplants demonstrates an intrinsic developmental program.Dev gram/?&? 1993;75: 105-112

82. Pederen EB, O'Keefe JA, Handa RJ, Castro AJ. Estrogen receptors arc present in neocortical transplants. J M?«m/7mmP/aj?1992;3:135-138

83. Paden CM, Gerlach JL, McEwen BS. Estrogen and progestin receptors appear in transplanted fetal hypothalamic preoptic area independently of the steroid environment. J M?«ro,rcH985; 5:2374-2381

84. SarM, Stumpf WE. Autoradiographic localization of radioactivity in the rat brain after injection of 1,2-3H-testosterone.EMocrmo/og}' 1973;92:251-256

85. Lieberburg I, MacLusky NJ, McEwen BS. 5-dihydrotestosterone receptors in rat brain and pituitary, gmm /?&? 1977; 100:598-607

86. Bonneau M, Ahdieh HB, Thornton JE, Peder HH. Cytoso-

lie androgen receptors in guinea pig brain and pituitary. *Brain Res* 1987; 413: 104-110

87. HandaRJ, Connolly PB, Resko JA. Ontogeny of cytosolic androgen receptors in the brain of the fetal rhesus monkey. *Endocrinology* 1988; 122: 1890-1896

88. Hcrbison AE. Neurochemical identity of neurons expressing estrogcn and androgcn receptors in sheep hypothalamus. *J Rep rod Eertil* 1995; 49 (Suppl): 271 -283

89. McGinnis MY, Davis PG, Mcancy MJ, Singer M, McEwen BS. *In vitro* measurement of cytosol and cell nuclear androgen receptors in male rat brain and anterior pituitary gland.*Brain* tfe.v 1983; 275: 75-82

90. Handa RJ, Reselli CE, Horton L, Rcsko JA. The quantitative distribution of cytosolic androgcn receptor in microdissected areas of male rat brain: effects ofestrogen treatment. *Endocrinology*- 1987; 121:233-240

91. Handa RJ, Reid DL, Rcsko JA. Androgen receptor in brain and pituitary of female rats: cyclic changes and comparison with the male. Mo/ *Reprod* 1986; 34: 293-303

92. Hicks LL, Walsh PC. A microassy for the measurement of androgcn receptor in human prostate tissue. *Steroids* 1979; 33:389-406

93. Wood RI, Brabec RK, Swann JM, Newman SW. Androgen and estrogen receptor containing neurons in chemosensory pathways of the male Syrian hamster brain. *Brain Res* 1992:596:89-98

94. Sheridan PJ. Unaromati/.ablc androgcn is taken up by the neonatal rat brain: two receptor systems for androgen. *Dev Neurosci* 1981; 4: 46-54

95. Kuiper GGJM, Faber PW, van Rooij HJC, van der Korput JAGM, Ris-Stalpus C, Klaasseen *Petal.* Structural organization of the human androgen receptor gene. *J* Mo/ *Endocrinol m9;2:* 11-14

96. Zhou Z. Lane MV. Kcmppaincn JA, French PS, Wilson EM. Specificity ofligand dependent androgen receptor stability: receptor domain interactions influence ligand dissociation and receptor stability. Mo/ *Endocrinol* 1995; 9:208-218

97. McLachlan RI, Temple BL, Miller MA, Bicknell JN, Bremmer WJ, Dorsa DM. Androgcn receptor gene expression in rat central nervous system: evidence for two mRNA transcripts. Mo/*CellNeurosci* 1991; 2: 1 17-122

98. Burgess LH, Handa RJ. Hormone regulation of androgen receptor messenger ribonucleic acid in brain and pituitary *of male rat. Mol Brain Res* 1993; 19:31-38

99. Price Jr-RH, Wilson ME, HandaRJ. A smaller form of androgen receptor mRNA in brain contains a truncated *5* - untranslated region. Joe *Neurosci Abstr* 1995; 351.11

100. Mizokami A, Chang C. Induction of translation by 5 untranslated region of human androgen receptor mRNA. *J Biol Chem* 1994; 269: 25655-25659

101. Hart BL. Neonatal dihydrotestosterone and estrogen stimulation: effects on sexual behavior of male rats. *Horn Behav* 1977; 8: 193-200

102. Beatty WW. Hormonal organization of sexual differences in play fighting and spatial behavior. Prog Dram *Res* 1984; 61: 320-324

103. Finney HC, Erpino MJ. Synergistic effect of estradiol benzoate and dihydrotestosterone on aggression in mice /form *Behav* 1976; 7:391 -400

104. Liebcrburg I, MacLusky NJ, Roy EJ, McEwen BS. Sex steroid receptors in the perinatal rat *brain. Brain* /4m *Zool* 1978; 18:539-544

105. Malshury CW, Babstock D. Ontogeny of androgen receptor immunoreactivity in rat brain. *Soc Neurosci Abstr* 1996; 22:558

106. Kashon ML, Hayes MJ, Shek PP, Sisk CL. Regulation of brain androgen receptor immunoreactivity by androgen in prcpubertal male ferrets. *Biol Reprod* 1995; 52: 1198-1205

107. Rodriguez EW, Burgess LH, Lubahn DB, French FS, Wilson EM, Handa RJ. Development of rat pituitary and hypo thalamic androgen receptor and AR mRNA. *Soc NeurosciAbst \990;* 144.6

108. McAbee M, Handa RJ, DonCarlos LL. Developmental profile of androgen receptor mRNA expression in the forebrain of peri natal *rats. Soc Neurosci Abstr 1995;* 423.13

109. McAbee M, DonCarlos LL. Hormonal regulation of androgen receptor (AR) mRNA expression in the neonatal rat forebrain. Joe *Neurosci Abstr* 1996; 22:558

110. Toyooka KR, Connolly PB, Handa RJ, Rcsko JA. Ontogeny of androgen receptor in fetal guinea pig brain. *Biol Reprod* 1987; 41:204-212

11. Shell SA, Kirn KL. Aromatase, 5oc-reductasc and androgen receptor levels in fetal monkey brain during eairly development. *Neuroeiidocrinology* 1990; 52: 94-98

12. Connolly PB, Handa R.T. Rcsko JA. Progesterone modulation of androgen receptors in the brain and pituitary of male guinea pigs. *Endocrinology* 1988; 122: 2547-2553

113. Quarmby VE, Yarbrough WG, Lubahn DB, French FS, Wilson EM. Autologous down-regulation of androgen receptor messenger ribonuclcic acid. *MolEndocrinol* 1990; 4:22-28

114. Kerr JE, Allore RJ, Beck SG, Handa RJ. Distribution and hormonal regulation of androgen receptor messenger ribonucleic acid in rat hippocampus. *Endocrinology* 1995; 136: 3213-3221

115. Abdelgabir SE, Connelly PB, Resko JA. Androgen regulation of androgen receptor mRNA differ in rat prostate and selected brain areas. *Mol CellNeurosci* 1993; 4: 532-537