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Cloning and In Situ Hybridization Analysis of Estrogen Receptor, Progesterone Receptor, and Androgen Receptor Expression in the Brain of Whiptail Lizards (*Cnemidophorus uniparens* and *C. inornatus*)

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ABSTRACT

Gonadal steroid hormones act upon specific areas of the vertebrate brain to affect the reproductive physiology and behavior of the animal. Steroid receptors are members of a superfamily of ligand-dependent transcription factors that mediate the effects of steroid hormones by modulating gene expression in the cells containing the receptors. The neuroanatomical distributions of steroid receptor-containing cells have been described for several species by using steroid autoradiography, immunocytochemistry, and more recently in situ hybridization. We have used the polymerase chain reaction to amplify and clone fragments of the estrogen receptor, progesterone receptor, and androgen receptor of whiptail lizards (genus *Cnemidophorus*). These clones were used to produce probes for use in in situ hybridization assays and to map the neuroanatomical distribution of all three sex steroid hormone receptors in the forebrains of unisexual (*C. uniparens*) and sexual (*C. inornatus*) species of whiptail lizards. The distribution of receptor-expressing cells reported here is in general agreement with previous reports in other species with receptor-containing cells concentrated in septal, amygdaloid, cortical, preoptic, and hypothalamic nuclei. © 1994 Wiley-Liss, Inc.

Key words: steroid receptor, hypothalamus, preoptic area, sexual behavior, reptile

Sex steroid hormones modulate the behavior and physiology of an animal by acting upon specific neuronal groups in the vertebrate brain via steroid-specific receptor proteins (Pfaff and Schwartz-Giblin, 1988). Steroid receptors are members of a superfamily of ligand-dependent transcription factors that mediate the genomic effects of gonadal steroids by altering the rate of gene expression (Evans, 1988). Sex steroid-induced changes in gene expression in certain brain nuclei are thought to influence directly the display of reproductive behaviors. For example, estrogenic induction of the neuropeptide oxytocin and its receptor in the ventromedial nucleus of the hypothalamus is thought to be crucial to the expression of lordosis behavior in female rats (Schumacher et al., 1989).

The distribution of sex steroid hormone receptors in the brain has been investigated in several species of vertebrates by utilizing steroid autoradiography (Morrell and Pfaff, 1978; Morrell et al., 1979; Kim et al., 1981; Halpern et al., 1982; Watson and Adkins-Regan, 1989; Lubischer and Arnold, 1990), immunocytochemistry (Warembourg et al., 1986; Gahr et al., 1987; Sterling et al., 1987; Blaustein et

al., 1988; Balthazart et al., 1992), and in situ hybridization (Simerly et al., 1990; Hagihara et al., 1992). A comparison of the results of these studies demonstrates that the distribution of the steroid receptor-containing neurons in the brain have been highly conserved throughout vertebrate evolution (Pfaff and Schwartz-Giblin, 1988). This conservation of receptor distribution may prove useful in identifying homologous neuronal groups in evolutionarily divergent taxa.

The primary goal of the present study was to validate a technique that will allow the analysis of steroid receptor expression in specific brain nuclei and to provide a precise neuroanatomical map of the neuronal groups containing receptors for estrogen, progesterone, and androgen in the whiptail brain. This information provides a necessary foundation for future investigations into the neural mecha-

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nisms controlling sexual and pseudosexual behavior in these species. We used reverse transcription in combination with polymerase chain reaction (PCR) to clone cDNA fragments of *Cnemidophorus* estrogen receptor (ER), progesterone receptor (PR), and androgen receptor (AR) and used the clones as probes in the in situ hybridization assays to detect cells expressing each steroid receptor. The results presented in this report provide the first documentation of the distribution of all three sex steroid receptors in the reptilian brain and should prove useful in identifying neuronal groups that mediate the effects of sex steroids on reproductive behavior and physiology in reptiles in general.

MATERIALS AND METHODS

Animals

C. uniparens were captured in and around Portal, Arizona; *C. inornatus* were captured in Texas, near Dryden and Sanderson. The lizards were transported to the University of Texas at Austin, where they were maintained as described previously (Wade and Crews, 1991). The brains of nine *C. uniparens*, nine female *C. inornatus*, and four male *C. inornatus* were used for ER and PR in situ hybridization. In addition, seven *C. uniparens* and two male *C. inornatus* brains were assayed for ER, PR, and AR on adjacent sections.

Cloning and sequencing

Primer sequences used for PCR amplification were chosen on the basis of sequence homology comparisons between published amino acid sequences for each receptor: ER—human (Green et al., 1986), chicken (Krust et al., 1986), rat (Koike et al., 1987); PR—rabbit (Loosfelt et al., 1986), chicken (Gronemeyer et al., 1987), human (Misrahi et al., 1987), and AR—rat and human (Chang et al., 1988). The position and orientation of the primers for each receptor are illustrated in Figure 1. The primer sequences are as follows: ER1 5' GG(AGT) CA(CT) AA(CT) GA(TC) TA(TC) ATG TG 3'; ER2 5' TCC AT(GT) CC(CT) TT(AG) TT(AG) CTC AT 3'; PR1 5' CCT GTG GAA GCT GTA AAG TCT TC 3'; PR2 5' GGT TTC ACC ATC CCT GCC A 3'; AR1 5' TG(TC) TA(TC) GA(AG) GCI GGI ATG AC 3'; AR2 5' CCA (IC)CC CAT NGC (AG)AA NAC CAT 3'; AR3 5' GCC ATC AT(CT) TCI GG(AG) AA(GA) 3', where bases in parentheses denote degenerate positions, I represents inosine, and N represents a degenerate position containing all four nucleotide possibilities.

For ER and PR amplification, cDNA was synthesized from random-primed total RNA extracted from *C. uniparens* oviductal tissue. For AR amplification, cDNA was synthesized from AR3-primed total RNA extracted from male *C. inornatus* kidney tissue. The receptor cDNAs were amplified by PCR and cloned into the pGEM7+ (Promega) plasmid for ER or the pCRII (Invitrogen) plasmid for PR and AR. The ER and PR clones were subsequently subcloned as indicated in Figure 1 to aid in sequencing and to provide fragments of suitable size for use as an in situ hybridization probe.

Probe preparation

Probes for in situ hybridization were prepared from LYCUER1b (372 bp), LYCUPR3a (394 bp), and GLCIAR1 (399 bp). Sense and antisense cRNA probes were synthesized by using SP6 or T7 RNA polymerase incorporating 35 S-UTP (NEN) at a specific activity of 9×10^8 cpm/ μ g probe. "Cold" antisense strands for competition controls were synthesized by incorporating 3 H-UTP (NEN) at a low specific activity (1,300 cpm/ μ g) to allow accurate quantification. The probes were then phenol/chloroform extracted and ethanol precipitated twice in ammonium acetate to remove unincorporated nucleotides. The purified cRNA probes were heat-denatured and added to the hybridization solution [50% formamide, 10% dextran sulfate, 0.3 M NaCl, 10 mM Tris HCl (pH 8.0), 1 mM EDTA, 1× Denhardt's solution, 10 mM dithiothreitol and 0.5 mg/ml tRNA] at a final concentration of 0.3 μ g probe \times length (kb)/ml. The competition control hybridization solutions also contained 100-fold excess of competing "cold" antisense strand.

Tissue preparation and hybridization

The animals were killed by rapid decapitation, and their brains were immediately removed and placed on dry ice and subsequently stored at -80°C until sectioning. Twenty-micrometer-thick coronal cryosections were melted on RNase-free polylysine-coated microscope slides and allowed to dry at room temperature before storing in slide boxes with desiccant at -80°C. Sections were placed on a series of slides so that adjacent sections could be hybridized to different probes. Slides containing control sections were treated with RNase A (20 μ g/ml) for 30 minutes prior to storage.

At the time of the assay, the tissue was fixed in 4% paraformaldehyde (pH 7.2) at room temperature for 20 minutes, rinsed 5 minutes in 3× phosphate-buffered saline

Abbreviations

AC	anterior commissure	MPA	medial preoptic area
ACC	nucleus accumbens	NS	nucleus sphericus
AH	anterior hypothalamus	NSA	nucleus septalis anterior
AMC	nucleus centralis amygdalae	NSL	nucleus septalis lateralis
AME	nucleus externus amygdalae	NSM	nucleus septalis medialis
AML	nucleus lateralis amygdalae	OC	optic chiasm
BST	bed nucleus of the stria terminalis	OT	optic tract
CXD	cortex dorsalis	PC	posterior commissure
CXL	cortex lateralis	PH	nucleus periventricularis hypothalami
CXM	cortex medialis	PP	nucleus periventricularis preopticus
DH	nucleus dorsalis hypothalami	PR	nucleus premammillary
DL	nucleus dorsolateralis anterior	SC	nucleus suprachiasmaticus
DM	nucleus dorsomedialis	SO	nucleus supraopticus
DVR	dorsal ventricular ridge	STR	striatum
I	nucleus interstitialis	TECT	optic tectum
LFB	lateral forebrain bundle	TS	torus semicircularis
LHA	lateral hypothalamic area	VE	ventricular ependymal organ
LPA	lateral preoptic area	VMH	nucleus ventromedialis hypothalami
LTP	lentiformis thalami pars plicta		

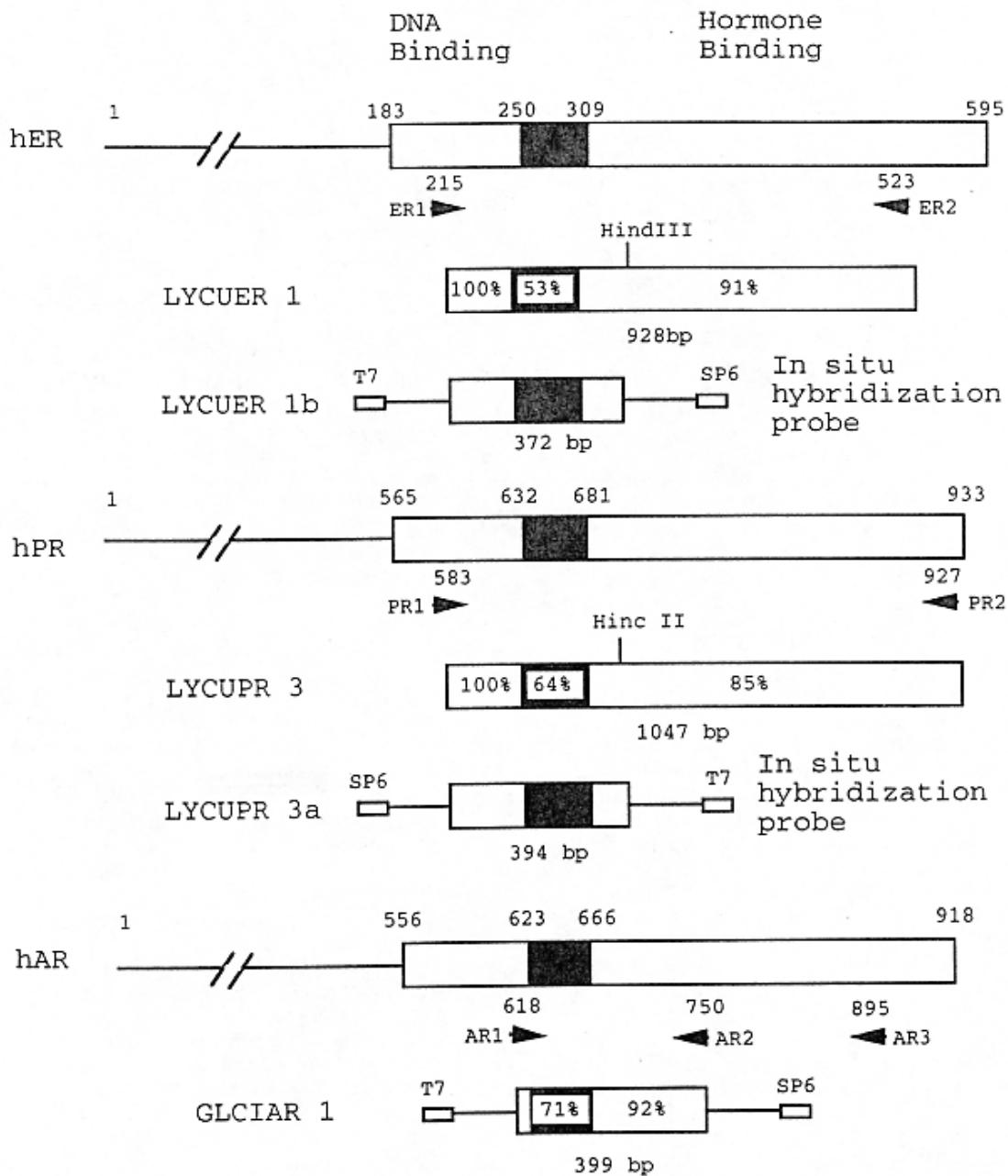


Fig. 1. Schematic diagrams showing the size, position, and sequence homology of the *Cnemidophorus* estrogen receptor (LYCUER1), progesterone receptor (LYCUPR3), and androgen receptor (GLCIAR1) clones relative to the human sequences (hER, hPR, hAR). The amino acid positions indicated above the diagrams are from Green et al. (1986) for the estrogen receptor, Misrahi et al. (1987) for the progesterone receptor, and Chang et al. (1988) for the androgen receptor. The exact position

of the primers used for polymerase chain reaction (PCR) are indicated by arrows. The amino acid sequence homologies of the functional domains of the lizard and the human receptors are indicated within the diagrams of the clones. LYCUER1 and LYCUPR3 were subcloned utilizing the Hind III and Hinc II restriction sites, respectively, as indicated. LYCUER1b, LYCUPR3a, and GLCIAR1 were used to generate the probes used in the in situ hybridization.

(PBS), followed by two 5 minute washes in 1× PBS. The sections were then dipped in 0.1 M triethanolamine (TEA; pH 8.0) followed by 10 minutes in freshly prepared TEA/0.25% acetic anhydride, rinsed in 2× SSC (2× SSC = 0.3 M sodium chloride, 0.03 M sodium citrate, pH 7), dehydrated in ascending ethanol, delipidated in chloroform, rinsed in 95% ethanol, air dried, and prehybridized overnight (50°C) in hybridization solution (see above) in which the probe was replaced with tRNA (2.5 mg/ml final concentration). Prehy-

bridization and hybridization took place in airtight chambers containing moistened paper towels. Prehybridization solution was rinsed off in 2× SSC, and sections were dehydrated in ascending ethanol and air dried prior to hybridization.

Hybridization solution was applied to the sections and hybridization proceeded overnight at 50°C. The sections were then washed twice for 15 minutes in 1× SSC, 50% formamide, 0.1% 2-mercaptoethanol (2-ME) at 50°C, then RNase A digested (20 µg/ml) for 30 minutes at 37°C, and

washed 30 minutes in 1× SSC, 0.1% 2-ME (50°C). Final high-stringency washes consisted of two 30 minute washes in 0.1× SSC, 1% 2-ME (50°C). The high-stringency washes for the thermal stability controls were carried out at 75°C and 85°C. The sections were then dehydrated in alcohols and air dried.

The slides were then dipped in Kodak NTB-2 autoradiographic emulsion, dried, and exposed at 4°C for 4 weeks, developed in Kodak D-19 developer, and fixed. After fixation, the sections were washed in water, stained with cresyl violet or thionin, and coverslipped using Permount.

Analysis

Sections were analyzed by darkfield microscopy. Specific labeling was defined as clusters of silver grains lying over cresyl violet- or thionin-stained cell somata at a density at least three times that of background. Specific binding was further verified by comparing antisense hybridized sections with sections hybridized to the sense strand probe. Nomenclature for telencephalic brain regions was adapted from Smeets et al. (1986) and that for diencephalic regions from Cruce (1974) and Smeets et al. (1986).

RESULTS

Cloning and sequencing

The amino acid sequence homologies of the *Cnemidophorus* cDNA clones with the corresponding human receptor sequences is indicated in Figure 1. The high degree of serial homology in the DNA- and steroid-binding domains of each lizard receptor with sequences of human receptors indicate that each of the clones is indeed a fragment of the respective steroid receptor gene.

In situ hybridization validation

The distribution of receptor expression was determined by comparison of sections hybridized to ^{35}S labeled antisense probe with sense strand hybridizations. Specific labeling was only slightly diminished after high-stringency washing at 75°C and 85°C and was eliminated by RNase pretreatment and by the presence of 100-fold excess "cold" antisense competitor probe. The PR and AR share a high degree of sequence homology in the DNA binding domain, which could result in some cross-reactivity in *in situ* hybridization experiments. However, comparison of labeled cell distribution for these receptors indicates that cross-reactivity is not a problem under the conditions used (Fig. 2).

Receptor distribution

The neuroanatomical pattern of gene expression was markedly different for each steroid receptor, and the pattern was consistent across all brains. No gross species or sex differences in expression patterns were found for any of the receptors. This is similar to previous studies in which no gross anatomical sex differences in the distribution of steroid receptors were found, although differences in the numbers of labeled cells and intensity of labeling have been reported (Lauber et al., 1991). The present experimental design is inappropriate for performing such quantitative analysis; therefore, quantitative species or sex differences were not examined. Figures 3–5 illustrate the sex steroid receptor distribution in selected sets of adjacent sections in the brain of *C. uniparens*.

Telencephalon. The anterior portion of the dorsal ventricular ridge (DVR) contained a group of cells expressing

AR, extending rostrally from the level illustrated in Figure 3A. No ER- or PR-containing cells were found in this region. In the cortex, ER-containing cells were found in the most medial and lateral portions of the dorsal cortex (Fig. 3B). Very light PR labeling was also detected in the dorsal cortex of some brains (Fig. 4B). The nucleus accumbens (ACC) contained both PR- and AR-labeled cells (Fig. 3B).

In the septum, each receptor had a distinct pattern of labeling (Figs. 3B,C, 4A–C). ER-labeled cells first appeared at a position rostral to the appearance of either AR or PR labeling (Fig. 3B,C). The AR probe labeled the densely packed cells in the nucleus septalis lateralis (NSL) adjacent to the lateral ventricle (Figs. 2C, 4C). In contrast, PR labeling was primarily located in the nucleus septalis medialis (NSM; Figs. 2B, 4A–C).

The nucleus externus amygdalae (AME) was labeled with the AR probe, but not with the ER or PR probes (Figs. 2, 3C, 4A). A few ER-expressing cells were found in the nucleus centralis amygdalae (AMC; Fig. 4A). Both the AMC (Figs. 2, 3C, 4A) and the nucleus lateralis amygdalae (AML; Fig. 2) were labeled with the PR probe.

Diencephalon. In the thalamus, the nucleus dorsolateralis anterior (DL) was labeled by the AR probe, but not by the ER or PR probes (Fig. 5A). The nucleus periventricularis preopticus (PP) and the medial preoptic area (MPA) contained ER-, PR-, and AR-labeled cells, although the patterns of expression differed for each receptor (Figs. 3, 6). The distribution of ER, PR, and AR were similar at the most rostral extent of the PP (Figs. 6B–D). At a more caudal level, the patterns of expression diverged. PR labeling was concentrated in the PP and the medial aspect of the MPA, whereas AR labeling was more laterally distributed in the MPA (Fig. 6G,H,K,L). At the most caudal extent of the MPA, ER expression was restricted to the cells lining the ventricle (Fig. 6J). The nucleus suprachiasmaticus (SC) was labeled with both the ER and the PR probes (Fig. 4B). The nucleus supraopticus (SO) was labeled for ER (Fig. 3C). PR labeling extended in a continuous pattern from the PP and the MPA through I, reaching the AMC (Fig. 2C). All three receptors were also present in the anterior hypothalamus (AH), although PR and AR labeling was much less intense compared to the MPA (Fig. 5A).

The nucleus ventromedialis hypothalami (VMH) and the nucleus periventricularis hypothalami (PH) showed similar patterns for each receptor (Figs. 5B, 7), with labeling being most intense on the lateral extent of the VMH and the ventral tip of the PH. ER and PR were expressed in the nucleus dorsalis hypothalami (DH) adjacent to the ventricular ependymal organ (VE), although ER labeling occurred in cells slightly more dorsal than the PR labeled cells. Progesterone receptor expression extended dorsally into the lentiformis thalami pars pecten (LTP; Figs. 5B, 7). A few lightly labeled PR- and AR-containing cells were located in the lateral hypothalamic area (LHA). The nucleus premammillary (PR) was labeled with the AR probe, but not by ER or PR probes (Fig. 5C).

Other areas. All three steroid receptors were found in cells in the optic tectum (Fig. 5B). The labeled cells in this area were continuous with cells in the torus semicircularis (TS), which contained ER and PR, but not AR (Fig. 5C). The hindbrain and brainstem nuclei were not analyzed.

DISCUSSION

The distribution of ER and AR expression in the *Cnemidophorus* brain is in general agreement with the steroid

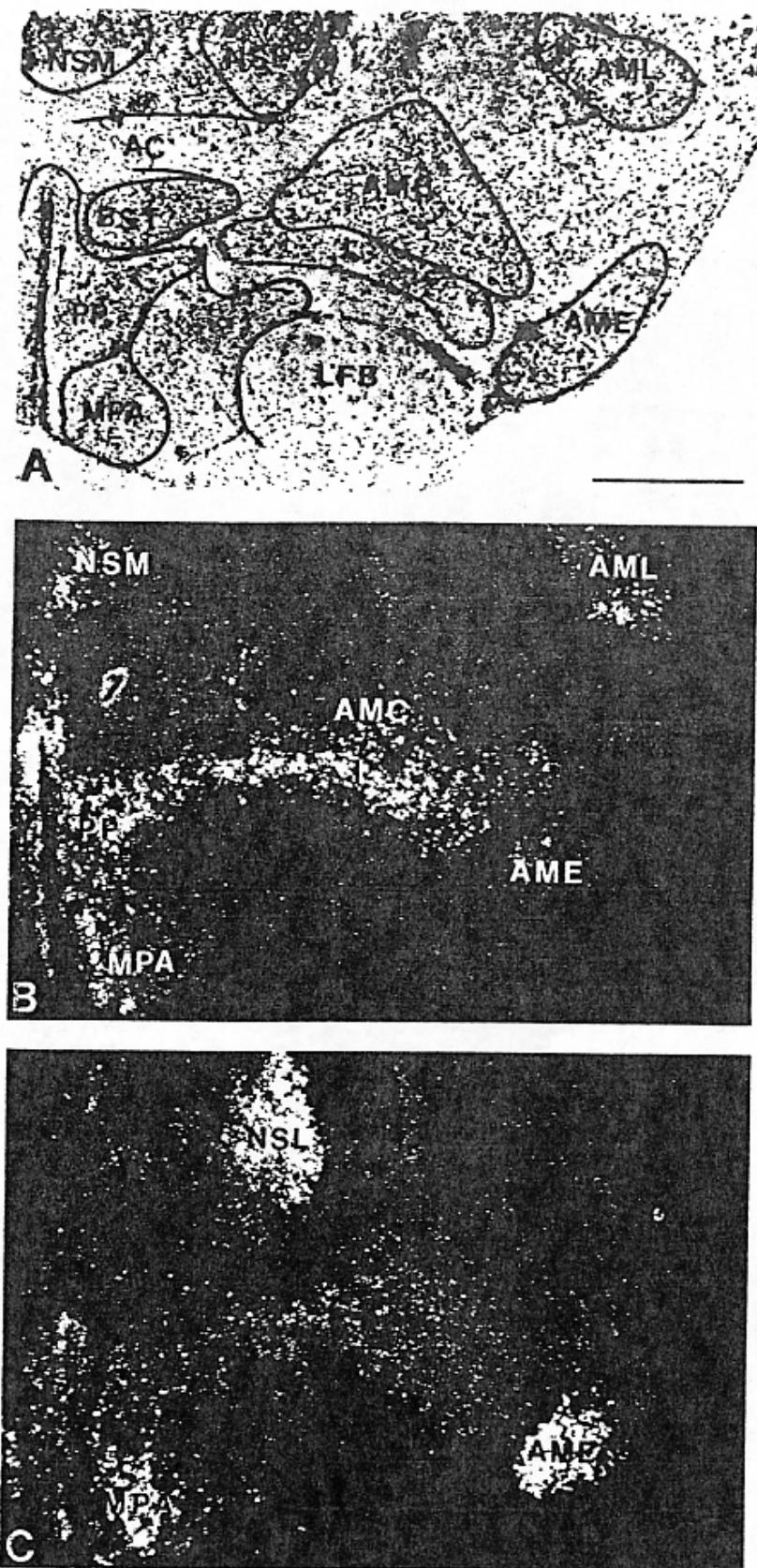


Fig. 2. Photomicrograph illustrating the different pattern of progesterone receptor (B) and androgen receptor (C) labeling in the brain of a male *C. inornatus*. A: Brightfield photomicrograph of thionin-stained section of the region shown in B and C. Scale bar = 500 μ m.

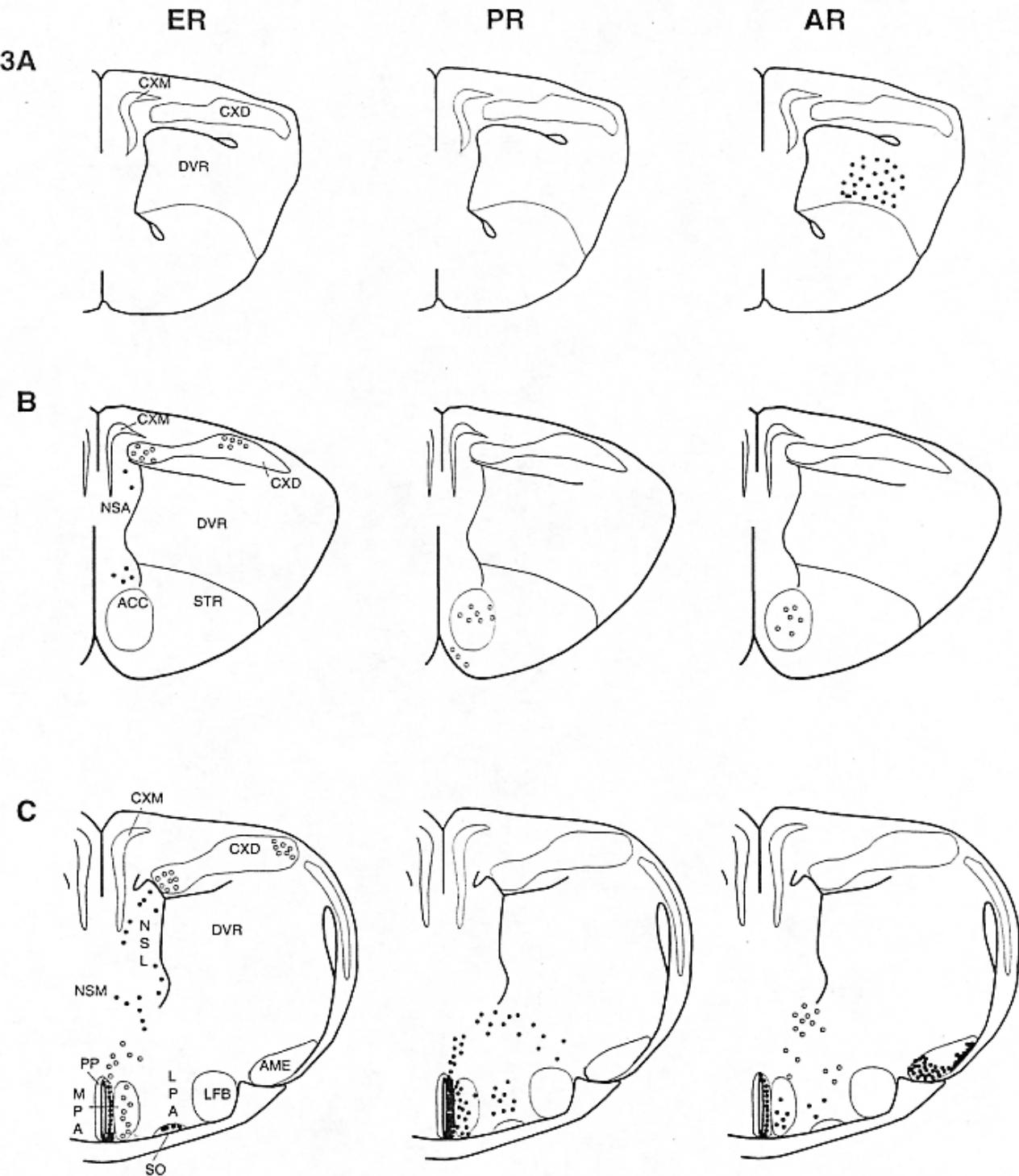


Fig. 3. A–C: Distribution of cells expressing steroid receptor mRNA in selected sections of the brain of a *Cnemidophorus uniparens*. Shown are the positions of cells expressing mRNA for estrogen receptor (ER; left column), progesterone receptor (PR; center column), and androgen receptor (AR; right column) in the right half of adjacent brain sections (20 μm apart). Solid circles indicate heavily labeled cells, and open circles indicate lightly labeled cells. See text for details.

uptake pattern reported in *Anolis carolinensis* (Morrell et al., 1979). However, some differences are apparent. In that study, the authors reported that the neuroanatomical topography of the estradiol- and androgen-concentrating

cells in the forebrain of *A. carolinensis* was virtually identical, with the exception of the pallium. In all other forebrain regions, estrogen-concentrating cells were more numerous than androgen-concentrating cells. Similar re-

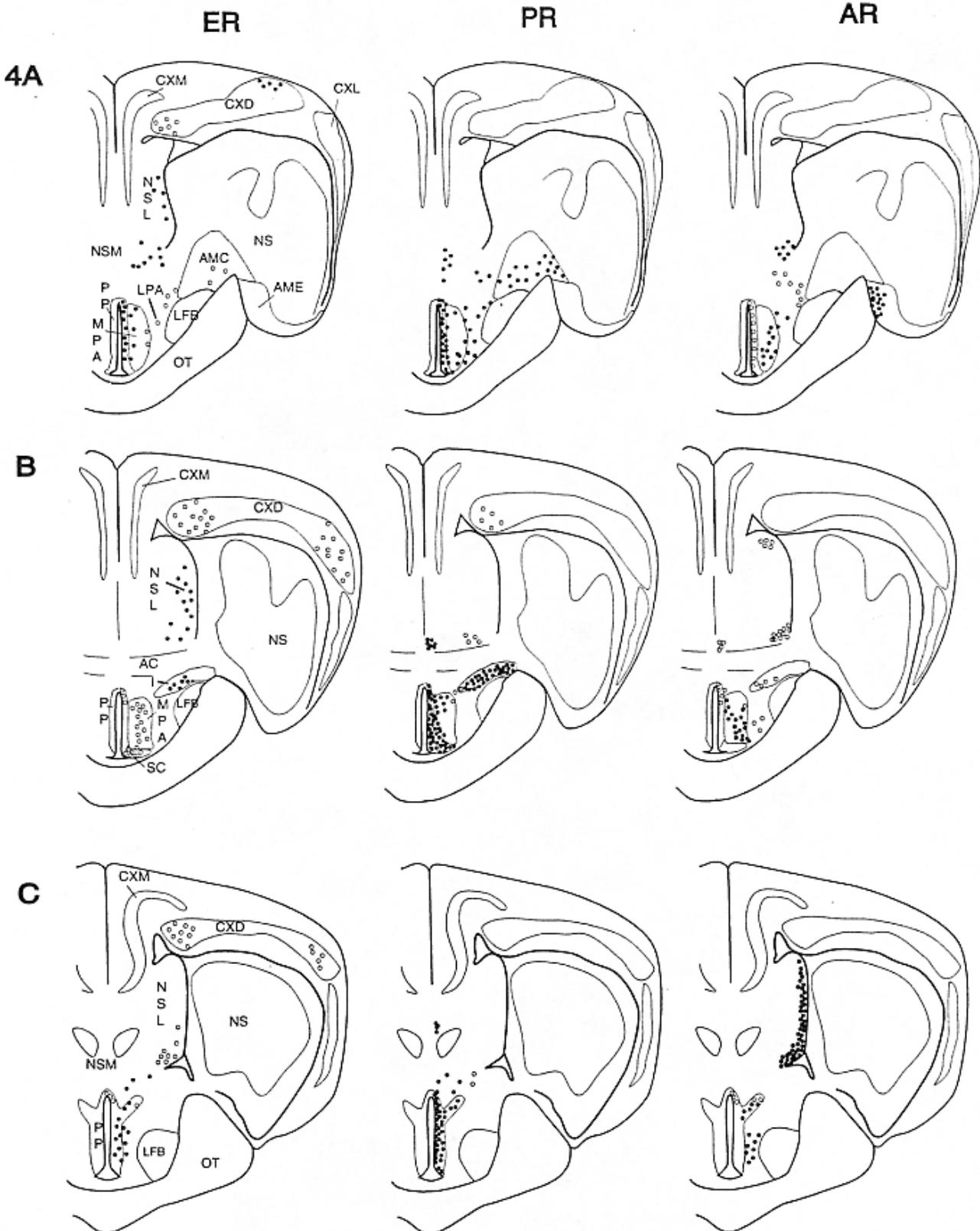


Fig. 4. A-C: See legend to Figure 3.

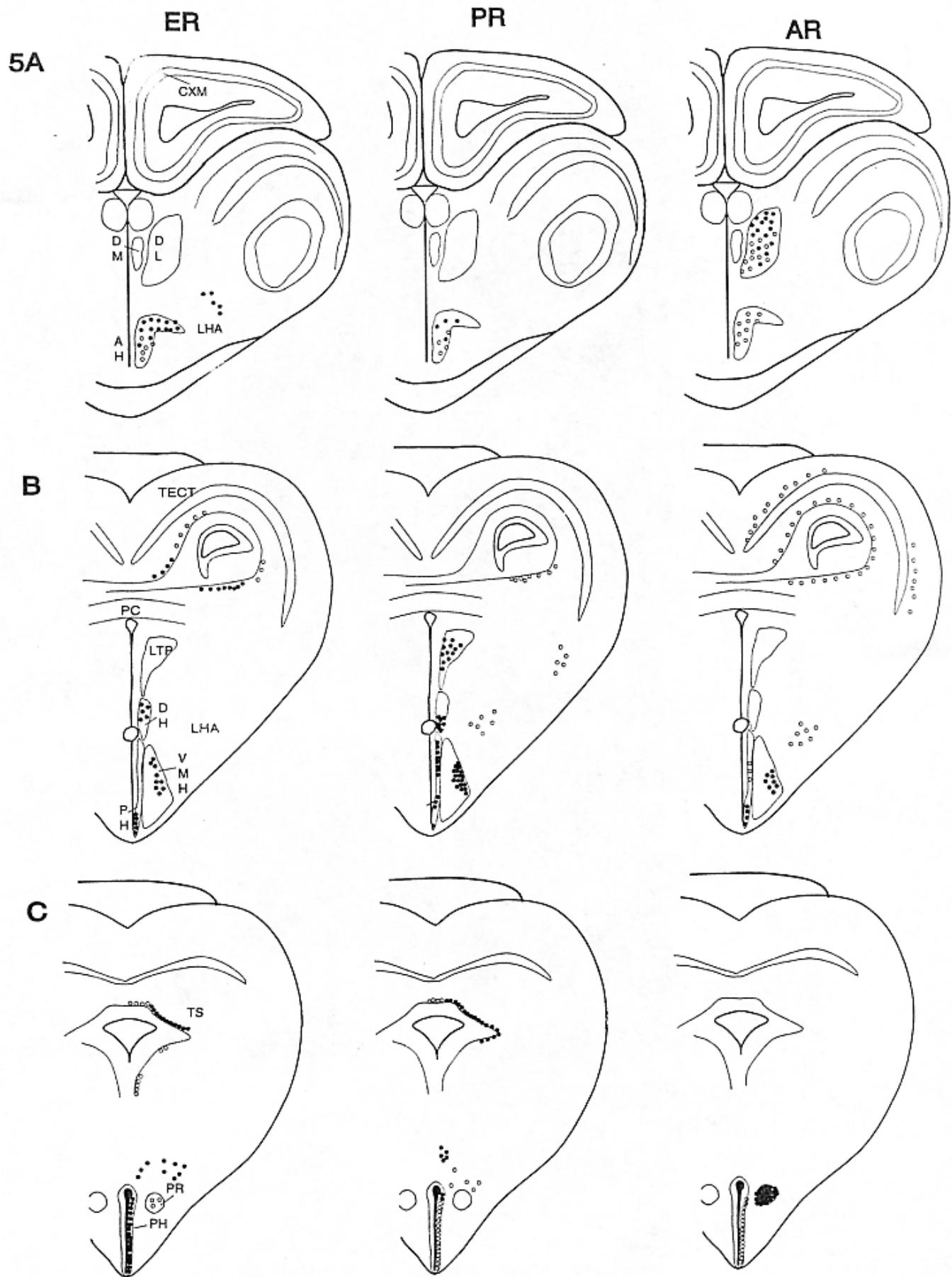


Fig. 5. A–C: See legend to Figure 3.

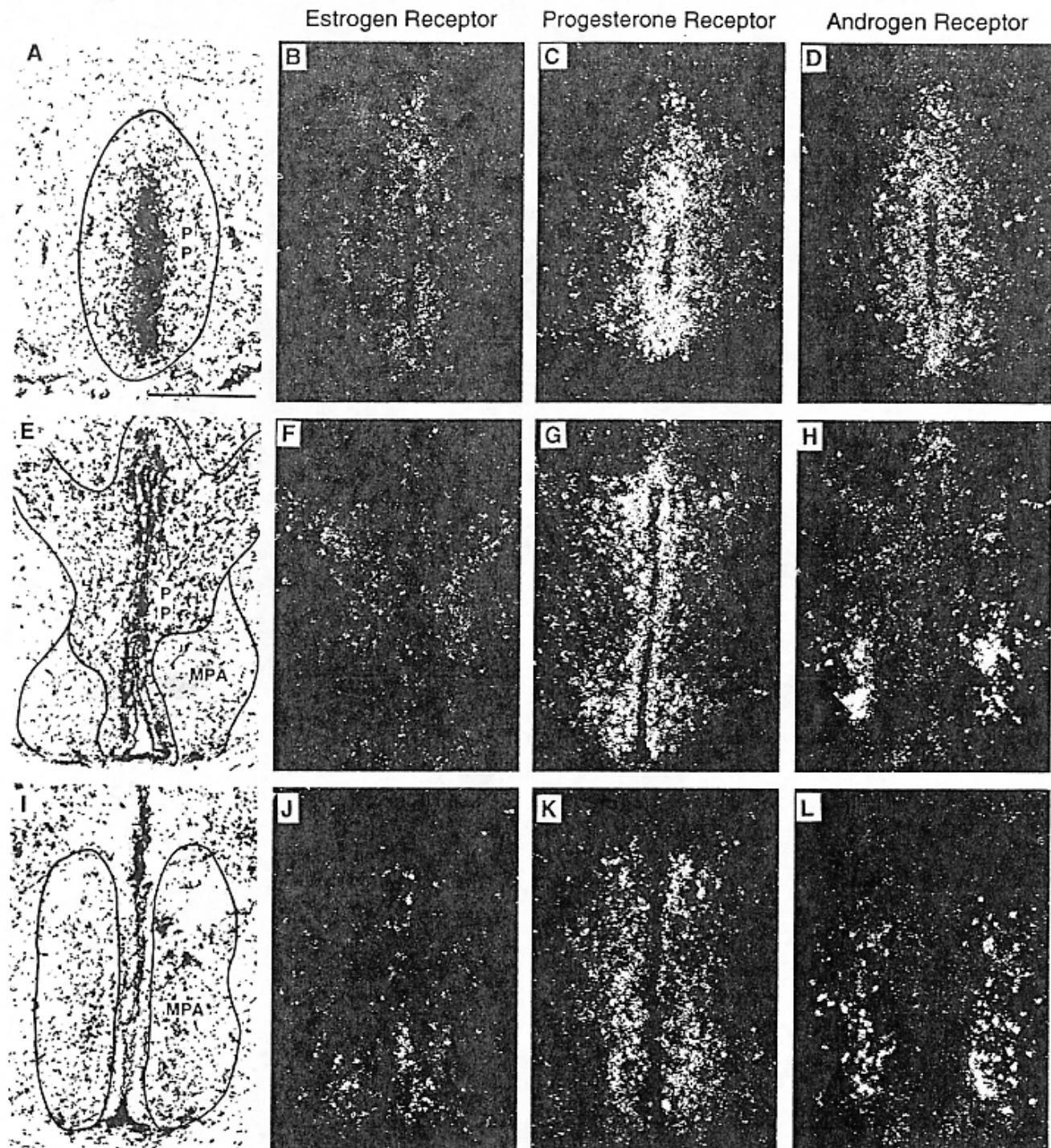


Fig. 6. Brightfield (left column; A,E,I) and darkfield (right three columns) photomicrographs illustrating the patterns of estrogen receptor (B,F,J), progesterone receptor (C,G,K), and androgen receptor (D,H,L) expression at different levels in the anterior diencephalon of *C. uniparens*. The top row is most anterior. Scale bar = 250 μ m.

sults were reported in garter snakes using the same technique (Halpern et al., 1982). Our results demonstrate several differences in ER and AR distribution in *Cnemidophorus*. For example, AR, but not ER or PR, was found in rostral extent of the dorsal ventricular ridge. AR antisense

probe heavily labeled many cells in the nucleus externus amygdalae, whereas no ER labeling was found in that area. We found many more heavily labeled AR-expressing cells than ER-expressing cells in the nucleus septalis lateralis, with ER-expressing cells in that nucleus being less densely

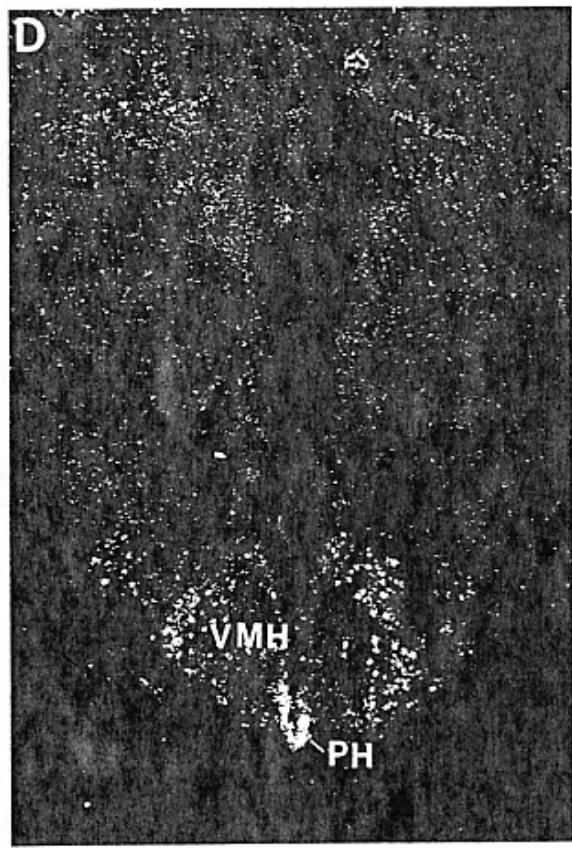
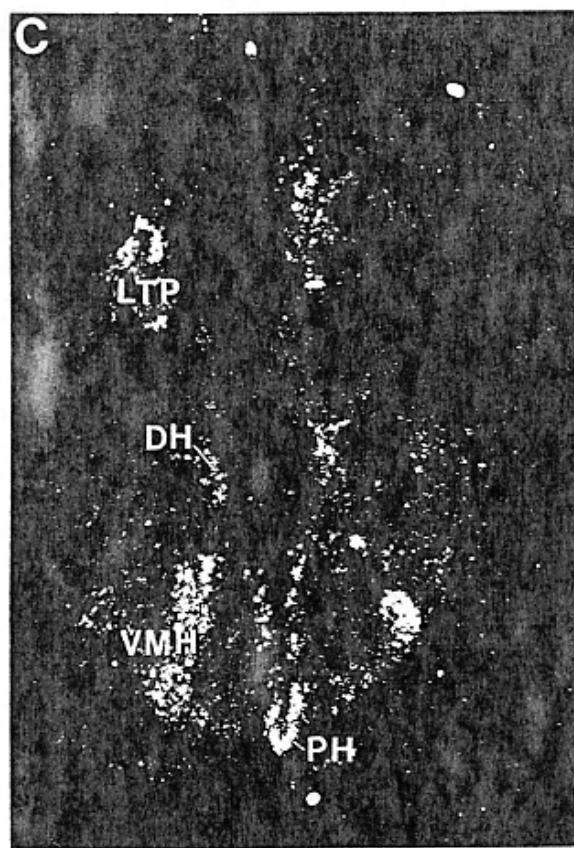
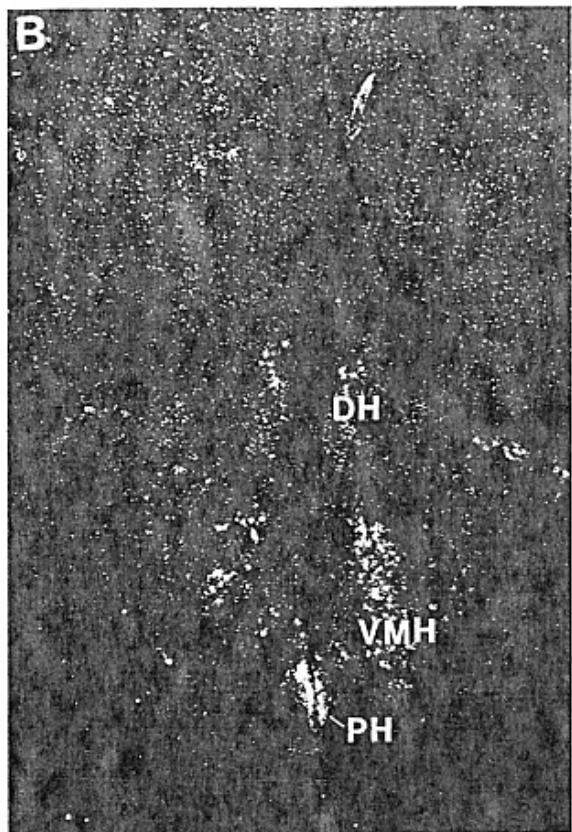
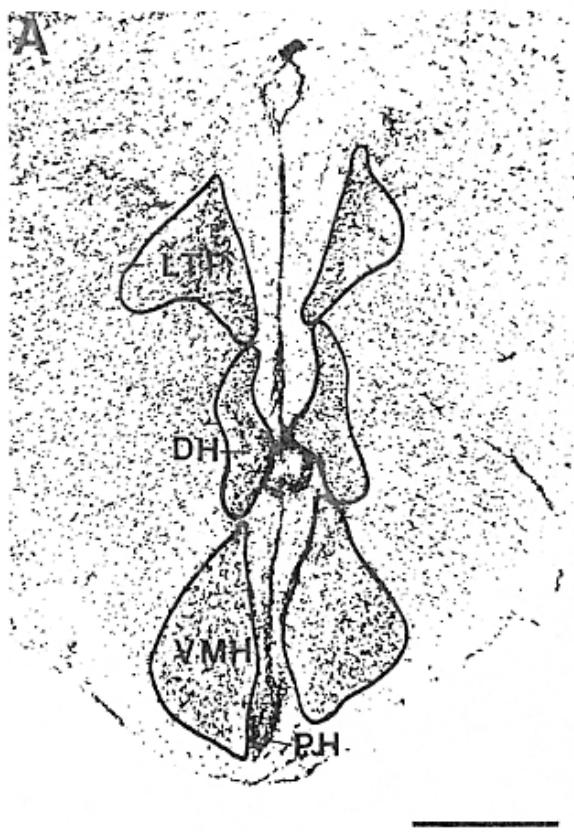


Fig. 7. Brightfield (A) and darkfield (B–D) photomicrographs illustrating the distribution of estrogen receptor (B), progesterone receptor (C), and androgen receptor (D) expression in the posterior diencephalon of *C. uniparens*. Scale bar = 500 μm .

packed and extending further rostrally than AR. We also found many AR-expressing cells in the thalamus and nucleus premammillary.

The differences in these results could be due to species differences, to differences between the two techniques, or both. For example, the tritiated testosterone used in the steroid uptake studies is likely to have been aromatized to estrogen and subsequently bound the ER. Because competitive binding experiments were not performed, it is also possible that the steroids may have bound heterologous receptors. Progesterone receptor distribution has not been previously reported in a reptile, although the presence of PR-containing cells in the hypothalamus has been demonstrated biochemically in *A. carolinensis* (Tokarz et al., 1981).

Because steroid autoradiography results are difficult to interpret due to problems discussed above, immunocytochemistry and *in situ* hybridization are the preferable techniques for detecting steroid receptors in the brain. Both techniques are very sensitive and exhibit a high degree of specificity. However, immunocytochemistry depends upon the recognition by the antibody of a particular amino acid sequence within the epitope, a condition often violated in comparative studies, because amino acid sequences are not always conserved in evolutionarily divergent species. Generation of species-specific antibodies is not feasible in most cases because large amounts of purified receptor are required. RT-PCR affords the opportunity to easily prepare species-specific cDNA clones that can be used as probes in *in situ* hybridization to analyze steroid receptor expression in the brains of atypical animal models.

Functional significance of the steroid receptor-containing neurons and comparisons with other vertebrates

Telencephalon

Cortex. ER was found to be localized in the medial and lateral extent of the dorsal cortex. Studies of the efferent connections of the dorsal cortex in *Gekko gecko* clearly demonstrate the limbic character of this region (Hoogland and Vermeulen-Vanderzee, 1989). In the gecko, this region has connections to the DVR, amygdala, septum, hypothalamus, and the striatum-accumbens area, each of which themselves contain steroid receptors in whiptail lizards. It has been suggested that the dorsal cortex is reptilian homologue of the mammalian ventral subiculum (Hoogland and Vermeulen-Vanderzee, 1989), a component of the hippocampal formation which contains ER and AR in rats (Simerly et al., 1990).

Dorsal ventricular ridge. The DVR is a major center for receiving auditory, somatosensory and visual information (Ulinski, 1983). The function of the region in lizards containing AR, but not ER or PR, is unknown, although a region of similar shape and position in the DVR of songbirds and quail has also been reported to contain AR but not ER or PR (Balthazart et al., 1992). This region, the magnocellular nucleus of the anterior neostriatum (MAN), is thought to play a role in the androgen-dependent acquisition and production of song in songbirds. The position of this region is comparable to a region shown to receive auditory thalamic projections in *Iguana iguana* (Foster and Hall, 1978). This suggests that this region may be involved in auditory processing, although why this area would be androgen sensitive in whiptails is unclear because there is no indication of vocal communication in these lizards. It is interesting that Moore and Crews (1986) reported that

female *C. inornatus* have uniformly low (i.e., below the detectability of the assay) levels of circulating androgens throughout their reproductive cycle, suggesting that this area may have a sexually dimorphic function, as does MAN in songbirds. Alternatively, it may be that the expression of AR in this area is generally conserved throughout evolution and may not function in species lacking vocal communication, but the steroid-dependent circuits remain present and can be utilized if vocal communication evolves. Some lizards have evolved rich vocal repertoires involved in sexual and other social behaviors (Marcellini, 1977).

Septum. Little is known about the reptilian septum; however, it is likely to be homologous to the septum of other vertebrates. The pattern of steroid receptor expression in the septum in reptiles is strikingly similar to that of both birds and mammals. In each case, AR and ER are concentrated in the lateral septum (Watson and Adkins-Regan, 1989; Simerly et al., 1990), whereas PR is restricted to the medial septal area (Lubischer and Arnold, 1990), consistent with the ideas that the steroid-dependent mechanisms in this area are evolutionarily ancient and that these cell groupings probably serve similar functions in reptiles, birds, and mammals.

Amygdala. The reptilian amygdala is thought to have functions similar to those of the mammalian amygdala, receiving olfactory input and mediating behaviors such as aggression (see Morrell et al., 1979). The most prominently labeled portion of the amygdala was the AME, which was labeled intensely with the AR probe. In birds, the nucleus taeniae (Watson et al., 1989), which lies in a position similar to that of the AME in lizards, the dorsolateral surface of the telencephalon, contains many AR cells, suggesting that these structures may be homologues. A similar structure also exists in the rat amygdala, the medial nucleus of the amygdala and the amygdalohippocampal area, also located on the dorsolateral surface of the telencephalon, are densely labeled with AR probe (Simerly et al., 1990). The function of the PR located in the AMC and AML of whiptails is unclear. It has been demonstrated that following ovulation when progesterone levels are elevated, receptivity ceases and female *C. inornatus* become very aggressive toward courting males (Lindzey and Crews, 1988). One or both of these areas may be involved in the mediation of progesterone-dependent sexual rejection and aggression following ovulation.

Diencephalon

Preoptic area and hypothalamus. The MPA and AH are known to be important in the steroid-dependent expression of masculine sexual behaviors in mammals, birds, and lizards (Sachs and Meisel, 1988). Intracranial implantation of androgen into the MPA and the AH of gonadectomized *C. uniparens* and male *C. inornatus* reinstates mounting and copulatory behavior (Mayo and Crews, 1987; Rozendaal and Crews, 1989), whereas lesioning of this area abolishes androgen-stimulated copulatory behavior (Kingston and Crews, 1994). In another lizard, *Anolis carolinensis*, intracranial implantation of estrogen in this region is also effective in stimulating courtship behavior in males (Crews and Mortengaler, 1979). Implantation and lesion studies are rather crude, and it is impossible to determine with certainty which cell groups in this general region are responsible for the stimulatory effect.

Male-like mounting and copulatory behavior in *C. uniparens* is regulated by progesterone rather than androgen (Grassman and Crews, 1986); androgen levels in the plasma

remain undetectable throughout the reproductive cycle (Moore et al., 1985b). The abundance of PR in the PP and the medial extent of the MPA suggests that these areas may be involved in mediating the progesterone-dependent expression of male-like pseudosexual behavior in *C. uniparens*, although intracranial implantation studies will be necessary to test this hypothesis. PR is also found in abundance in the MPA and PP of both birds and mammals. It has recently been suggested that progesterone may also have a facilitatory function in the activation of male sexual behavior in rats as well (Witt et al., 1994).

Supraoptic nucleus. The SO in *A. carolinensis* has recently been shown to contain arginine vasotocin (AVT), a neuropeptide involved in ovulation in reptiles. The concentration of AVT within the SO varied with ovarian state, with the concentration being highest in animals with large vitellogenic follicles (Propper et al., 1992). The authors suggested that changes in plasma estradiol concentrations may be responsible for the changes in AVT levels in the SO. The presence of ER within the SO of *Cnemidophorus* supports that hypothesis and likely mediates the elevation of AVT in the SO during follicular development. The function of the AVT in SO in reptiles is not known.

Ventromedial nucleus of the hypothalamus. The VMH is known to be involved in the estrogen-dependent mediation of receptive behavior in *C. inornatus* and *C. uniparens* because intracranial implantation of estrogen into this region facilitates receptive behaviors (Wade and Crews, 1991). The lateral portion of the VMH was heavily labeled for ER, PR, and AR. A similar pattern of receptor distribution is found in birds and mammals; in each case only the lateral portion of this nucleus contains labeled cells. In rats, estrogen and progesterone act sequentially in the VMH to facilitate lordosis (receptivity; Sachs and Meisel, 1988). Progesterone also acts synergistically with estrogens to facilitate receptivity in female *A. carolinensis* (McNicol and Crews, 1979; Wu et al., 1985). However, it is not known whether a similar mechanism exists in *Cnemidophorus*, although it is likely given the presence of PR in the VMH. The function of AR in the VMH is not known, though it is present in birds (Balthazart et al., 1992) and mammals (Simerly et al., 1990) as well. The volume of the VMH in *C. inornatus* has recently been demonstrated to be sexually dimorphic, with the VMH of males having a smaller volume than that of females (Crews et al., 1990). Furthermore, castration results in an increase in the volume of the VMH, and exogenous androgen treatment decreases the VMH volume in males (Wade et al., 1993). The AR in the VMH may function to mediate this hormone-dependent sexual dimorphism.

The nucleus premammillary is densely labeled with the AR probe. In the rat, the ventral premammillary nucleus is also well labeled with an AR probe but only slightly labeled with an ER probe (Simerly et al., 1990), indicating a possible homology between these areas.

Torus semicircularis. The torus semicircularis is well labeled with both ER and PR probes. These cells are continuous with neurons in the optic tectum which contain all three receptors. The TS is thought to be involved in auditory processing in lizards (Foster and Hall, 1978), and it has been suggested that this area is homologous to the TS of fish and amphibians, the nucleus intercollicularis of birds, and the inferior colliculus of mammals (see Morrell et al., 1979). The similarity of steroid receptor expression patterns supports this hypothesis. In birds, the nucleus

intercollicularis, which is similar in position to the labeled cells extending into the optic tectum in the lizard, is thought to be important in the hormone-induced activation of vocalization, such as bird song. The function of this region in whiptail lizards is unclear because vocalizations are not apparent. Nevertheless, this steroid receptor-expressing region in the reptile, like the AR-containing cells in the DVR, may be the evolutionary antecedent to an important part of the avian song system.

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