The Evolution of Neuroanatomical Substrates of Reproductive Behavior: Sex Steroid and LHRH-Specific Pathways Including the Terminal Nerve[1]

Leo S. Demski

*Physiology Group, School of Biological Sciences, University of Kentucky,
Lexington, Kentucky 40506*

SYNOPSIS. Fairly recent anatomical methods have made possible the mapping of neurobehavioral systems involving two types of reproductive hormones, gonadal steroids and the peptide luteinizing hormone releasing hormone (LHRH). Brain sites of steroid uptake are detected using autoradiography; LHRH is localized in cells and fibers using immunocytochemical procedures. Both hormone types are known to strongly influence sex behavior and it can reasonably be assumed that these effects are mediated in large part via systems identified using the anatomical procedures. Analysis of the comparative anatomy of these systems should therefore provide information useful in the construction of models concerning the evolution of neurohormonal control of reproductive behavior. The results of such a study are reported. Sex steroid and LHRH systems in cyclostomes, teleosts, amphibians, reptiles, birds and mammals are considered in detail. A synthesis of this information has led to the following ideas. Androgenic control of male reproductive systems has evolved in a number of nonhomologous motor systems controlling male reproductive behavior. Sex steroid and LHRH systems may interact at several different levels of the neuraxis but the most obvious overlap of the systems occurs in the septal and POA areas. The latter especially is a fairly constant and perhaps primitive feature. LHRH secretion into the systemic circulation was most likely the earliest means for LHRH modulation of both pituitary function and neural systems controlling reproductive behavior. Pathways for more direct delivery of LHRH to pituitary cells and brain nuclei probably developed in the early gnathostomes. The terminal nerve appears to be a rather conservative LHRH-containing pathway connecting olfactory systems with septal-preoptic nuclei. A function in pheromonal control of sex behavior is suggested. The general distribution of steroid concentrating cells and LHRH pathways in tetrapods seems to be rather constant. Absence of the systems in neocortical areas and their homologs is conspicuous.

INTRODUCTION

The neural systems controlling sexual behavior have been classically studied using lesion, stimulation and recording techniques (see details in Kelley and Pfaff, 1978; Pfaff, 1980). More recently, two anatomical procedures have been used to rapidly map functional pathways often extending over most of the neuraxis. The first method determines sites of action of gonadal steroids, hormones known to influence both the development and expression of sexual activity (see above references), by autoradiographic identification of tritium-labeled hormones concentrated in brain cell nuclei (see Stumpf, 1970a; Morrell and Pfaff, 1981). A second technique uses immuno-cytochemistry to detect luteinizing hormone releasing hormone (LHRH) in neuron cell bodies and axons (Barry, 1979). The peptide facilitates reproductive behavior in a variety of species including fishes (Demski et al., 1982), amphibians (Kelley, 1982; Moore et al., 1982) and mammals (Sakuma and Pfaff, 1980, 1983; Moss et al., 1979).

The anatomical methods have provided considerable data on brain pathways mediating sexual responses. While by no means "complete," these data are sufficient to permit some detailed comparisons among most vertebrate groups and from this, the suggestion of possible evolutionary trends in sex hormone-specific pathways. The results of such an analysis are reported in this paper. Interactions between the neural substrates for the two hormones may be especially important in the normal control of sexual activity since LHRH effects on reproductive behavior

appear to be modulated by gonadal steroids (Moss *et al.*, 1979; Shivers *et al.*, 1983). For this reason, comparisons of overlapping substrates for the two hormones have been emphasized. Details of the structure and connections of the terminal nerve (TN), an LHRH-containing pathway recently implicated in the pheromonal triggering of sexual responses (Demski and Northcutt, 1983) have also been stressed.

For simplicity, differences between androgen and estrogen systems have generally not been considered but rather, data for all sex-steroids in both males and females were pooled in making the final determinations (see Table 1). The results of hormone-mapping experiments are presented within separate phyletic groups, *e.g.*, cyclostomes, amphibians, etc.; certain major taxa are not included because of insufficient data, *e.g.*, elasmobranchs. The results for all groups are summarized, compared and discussed in the context of possible trends in the evolution of neural substrates of sexual behavior (see Conclusions).

STEROID-CONCENTRATING AND LHRH-CONTAINING SYSTEMS IN THE BRAINS OF VERTEBRATES

Cyclostomes

Estrogen-concentrating neurons have been identified in the forebrains of larval marine lampreys, *Petromyzon marinus* (Kim *et al.*, 1981*b*) and adult river lampreys, *Ichthyomyzon unicuspis* (Kim *et al.*, 1980). Although fewer hormone-concentrating cells were found in the larval forms, the distributions in both cases are similar. Labeled cells are located in the ventral periventricular areas of the telencephalon (anterior olfactory nucleus and corpus striatum), regions most likely homologous, at least as cell fields, to portions of the septal nuclei, amygdala, striatum, nucleus accumbens and the bed nucleus of the diagonal band in tetrapods (Northcutt, 1981; personal communications; see Table 1). Labeled cells are also present in the nucleus ventralis hypothalami (here considered homologous to the tuberal area, Table 1), ventral and dorsal thalamus and throughout the rostrocaudal extent of the preoptic area (Fig. 1).

With regard to LHRH in lampreys, immunoreactive perikarya have been located in the preoptic area (POA) in larval forms and both reproductive and nonreproductive adults. In the western brook lamprey, *Lampetra richardsoni*, cells in the posterior preoptic nucleus (PON) were stained in individuals in all three developmental stages (Crim *et al.*, 1979*b*); however, more reactive cells were found in the adults with most intense staining in the reproductive animals. In addition, immunoreactive neurons were found throughout the PON in only the adult stages. The cells include two classes of presumed CSF-containing neurons (Crim *et al.*, 1979*a*). In all stages, stained fibers pass from the PON to the neurohypophysis. The latter structure was heavily stained in adults and slightly stained in larvae. Similar LHRH-immunoreactive cells are located throughout the PON of spawning anadromous Pacific lamprey *Entosphenus tridentata* (Fig. 1). As in brook lamprey, fibers from PON cells sweep ventrally and enter the neurohypophysis which is heavily stained. Presumably, LHRH is released into blood vessels of the neural lobe and thereby affects reproductive functions via distribution in the systemic circulation (Crim *et al.*, 1979*a*; personal communications). Recent evidence indicates that LHRH analogs do indeed have profound effects on estradiol levels and ovulation in lampreys (Sower *et al.*, 1982). This distribution of LHRH-stained pathways has been confirmed in *E. japonica* (Nozaki and Kobayashi, 1979, 1980). Attempts to map LHRH systems in the brain of hagfish have so far been consistently negative (Crim *et al.*, 1979*a*; Nozaki and and Kobayashi, 1979, 1980).

Estradiol-concentrating neurons and LHRH-immunoreactive cells appear to overlap in their distribution in the PON (Table 1; Fig. 1). This contiguity may reflect mechanisms for steroid control of LHRH release. Whether or not the steroid-concentrating and LHRH-containing cells are identical has not been determined (see discussion in Conclusions).

The presence of a TN in agnathans has been suggested (Van Wijne, 1919; Ariëns Kappers *et al.*, 1936) but this interpretation

TABLE 1. *Distribution of LHRH and gonadal steroid-concentrating systems in the vertebrate CNS*

	Cyclo-stomes	Tele-osts	Amphib-ians	Rep-tiles	Birds	Mam-mals
Terminal nerve		L[a]				L
Olfactory bulb		L	l	l	L	L/s
Acc. olfactory bulb						L
Olfactory tubercle (Vl)[b]	s[a]	L	L	s	l	L/S
Paraolfactory lobe (birds)					l	
Lateral pallium (piriform ctx; Dp)		l		s		s
Dorsal pallium				S		
Cingulate cortex						L/s
Dorsal ventricular ridge (reptiles)				s		
Medial pallium (hippocampus; Dl-p, Dl-v)		l		S	S	s
Amygdala (archistriatum; Vc, Vi, Vs, Vp, NT)	s	l/S	S	S	S	L/s
Striatum (paleostriatum; parts of Dc and Dm)	s	L	S	S	S	
Neostriatum; hyperstriatum					S	
N. accumbens; diag. band; st. terminalis	s		L/S	S	l/S	L/S
Medial septum (Vv)	s	L/S	L	L/s	L/s	L/s
Lateral septum (Vd)	s		S	S	l/S	S
Preoptic area-ant. hypothalamus	L/S	L/S	L/S	L/S	L/S	L/S
Infundibulum-tuberal n.	L/S	L/S	L/S	L/S	l/S	L/S
Median eminence			L	L/S	L	L/S
Neurohypophysis	L	L	L			
Med. hypothalamus (perivent. and ventromed. n.)		L/S	L	S	L/S	L/S
Organum vasculosum lamina terminalis	L	L		L	L	L
Subfornical organ						L/S
Subcommissural organ					L	s
Pineal		l				
Habenula; stria medullaris		L	l		L	L/S
Thalamus	S	L/S	l/S	S	S	l/S
Retina; optic nerves		L				
Optic tectum		L/s	l	l/s	l	L/s
Torus semicircularis		L/S	l/S	S		
Central grey			l	S	L/S	L/S
Midbrain tegmentum		L	l	l/S	L/S	L/S
Pontomedullary tegmentum		L/S	l/S	l/S	S	L/S
Cerebellum		l				s
Spinal cord (motor)		S	S			S
Spinal cord (nonmotor)				S	S	S
Spinal cord (undetermined)		l	l			

[a] L and l, LHRH immunoreactive perikarya, fibers or terminals; S and s, gonadal steroid-concentrating cells; capital letters indicate that hormone is found in many species or at least its presence is well documented in one or more animals; lower case letters indicate that the substance is 1) weakly present in a few cases, 2) reported to be present but the data are somewhat ambiguous or not convincing or 3) reported to be present but the data are in abstracts or unpublished manuscripts. See text for references.

[b] Terms in ( ) represent either alternate names for a structure or abbreviated designations of its probable homolog in teleosts. Homologies for telencephalic areas are those proposed by Northcutt and Braford (1980) and Northcutt (1981). Abbreviations for telencephalic structures in teleosts: Dc, central zone of area dorsalis; Dm, medial zone of area dorsalis; Dl-p, posterior part of lateral zone of area dorsalis; Dl-v, ventral part of lateral zone of area dorsalis; Dp, posterior zone of area dorsalis; NT, nucleus taenia; Vc, commissural nucleus of area ventralis; Vd, dorsal nucleus of area ventralis; Vi, intermediate nucleus of area ventralis; Vl, lateral nucleus of area ventralis; Vp, postcommissural nucleus of area ventralis; Vs, supracommissural nucleus of area ventralis; Vv, ventral nucleus of area ventralis.

is based on several assumptions concerning the status of the "apical nerve" of *Amphioxus*. Until modern studies are carried out, *e.g.*, LHRH immunohistochemistry, it is not possible to assume with any assurance that the TN or its primitive homolog is present in either lampreys or hagfish.

Teleosts

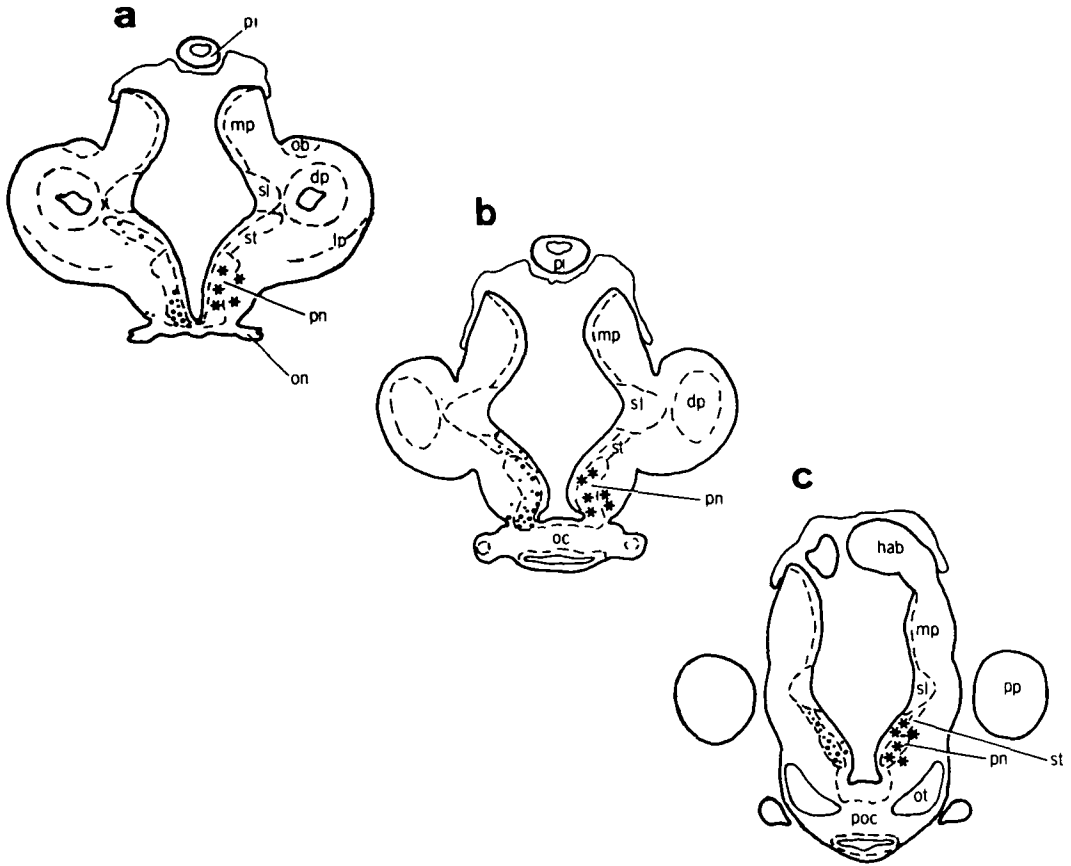Sex steroid-concentrating cells have been identified using autoradiographic proce-

FIG. 1a–c. Transverse sections of the forebrain of the adult river lamprey *Ichthyomyzon unicuspis* (redrawn from Figs. 7–9, Kim *et al.*, 1980). Section A is the most rostral. The size and density of solid dots on the left side of the figures represent the frequency and intensity of autoradiographic labeling of brain cell nuclei following administration of tritium-labeled estradiol to river lampreys (from Kim *et al.*, 1980). Asterisks on the right side of the sections indicate the position of perikarya stained following the application of anti-LHRH sera to brains of adult Pacific lampreys, *Entosphenus tridentata* (redrawn after Crim *et al.*, 1979a). Note especially the apparent overlap of both hormone-specific systems in the preoptic area. This comparison is of course only valid to the extent that species differences in the neural substrates for the hormones are not significant. Telencephalic areas were named using Northcutt's (1981) terminology; other structures are labeled as in Kim *et al.* (1980). Abbreviations: dp, dorsal pallium; hab, habenula; lp, lateral pallium; mp, medial pallium; ob, olfactory bulb; oc, optic chiasma; on, optic nerve; ot, optic tract; pi, pineal; pn, preoptic nucleus; pp, posterior pole of telencephalon; sl, submedial lobe; st, striatum.

dures to map the brains of five teleosts, *i.e.*, testosterone uptake in male green sunfish, *Lepomis cyanellus* (Morrell *et al.*, 1975a; Demski, 1978), male paradise fish, *Macropodus opercularis* (Davis *et al.*, 1977) and male and female toadfish, *Opsanus tau* (Fine *et al.*, 1982) and estradiol uptake in male paradise fish (Davis *et al.*, 1977) and both male and female goldfish, *Carassius auratus* (Kim *et al.*, 1978) and platyfish, *Xiphophorus maculatus* (Kim *et al.*, 1979). With the

exception of the more recent toadfish study, data from these investigations have been standardized and assembled in tabular form using a single anatomical nomenclature (*cf.*, Table 1 in Demski and Hornby, 1982). The information (see below) was utilized in compiling Table 1 of this paper.

In the forebrain, label is accumulated adjacent to the telencephalic ventricle just rostral (area ventralis telencephali pars ventralis, Vv) and immediately dorsal to
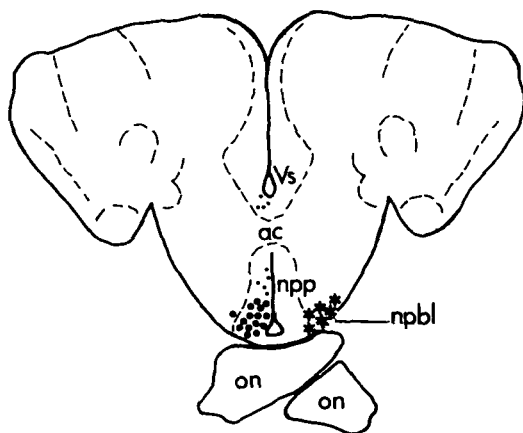
FIG. 2. Transverse section through the telencephalon of the platyfish, *Xiphophorus maculatus* (redrawn after Kim *et al.*, 1979). Size and density of solid dots (left side) represent the intensity and frequency of autoradiographic labeling of neuronal nuclei following administration of tritium labeled estradiol in *X. maculatus* (Kim *et al.*, 1979). Asterisks (right side) indicate the locations of cells stained following treatment of sections of brains of platyfish hybrids (*X.* sp.) with LHRH anti-sera (after Münz *et al.*, 1981). Note the partial overlap of the hormone-specific systems in the preoptic area. Terminology adapted from: Kim *et al.*, 1979; Münz *et al.*, 1981; Northcutt, 1981; and Peter and Gill, 1975. Abbreviations: ac, anterior commissure; on, optic nerve; npbl, nucleus preopticus periventricularis; Vs, area ventralis telencephali pars supracommissuralis.

the anterior commissure (area ventralis telencephali pars supracommissuralis, Vs in Fig. 2). These regions have been considered homologous to portions of the tetrapod medial septal nucleus and amygdala respectively (Northcutt and Braford, 1980; Northcutt, 1981). Further caudally, steroid-concentrating cells are located in the periventricular part of the POA (Fig. 2), the lateral tuberal nuclei, dorsal hypothalamus and the nucleus of the lateral recess. Other structures with some labeled cells include periventricular thalamic areas, the subpreglomerular region of the diencephalon and the nucleus of the tractus saccus vasculosus.

Several additional areas for testosterone uptake have been recently identified in the toadfish, *Opsanus tau* (Fine *et al.*, 1982). This species is well-known for producing courtship sounds which appear to have several androgen sensitive parameters. Testosterone-concentrating neurons were found in two small tegmental nuclei, one in the posterior medulla ventrolateral to the sonic motor nucleus (n. ventrolateralis medullae) and another in the dorsal medulla at cerebellar levels (n. periventricularis medullae) but not in the sonic motor nucleus itself. The positions of these nuclei correspond roughly to the predicted levels of the call pattern generators (Demski *et al.*, 1973; Demski, 1981) and may therefore be substrates for normal hormonal modulation of the call. Testosterone-concentrating cells are also localized in the torus semicircularis, an acoustic information processing center and region from which sounds can be evoked by electrical stimulation (Demski and Gerald, 1974; Fine, 1979; Demski, 1981). A few labeled cells were also found in the optic lobes or tectum. Thus, a system for sonic control in toadfish appears to be influenced by androgens at several critical levels. Analogous patterns have also been observed in tetrapods (see below).

LHRH-immunoreactive systems have been studied in several teleosts. Although there appear to be some differences in the distribution of reactive perikarya and fibers, four nuclei and their fiber connections seem to be characteristic for the group. The most rostral LHRH-containing cell bodies are located in the ventral parts of the olfactory nerve and bulbs in the goldfish (Stell *et al.*, 1984), a species with long olfactory tracts, and in the ventral telencephalon at the caudal border of the olfactory bulbs in several species with short (non-pedunculated) olfactory tracts including: the platyfish, *Xiphophorus* sp. (Schreibman *et al.*, 1979; Münz *et al.*, 1981, 1982; Halpern-Sebold and Schreibman, 1983); the bluegill sunfish, *Lepomis macrochirus* (Münz *et al.*, 1982), the cichlid, *Cichlasoma biocellatum* and the eel, *Anguilla japonica* (Nozaki and Kobayashi, 1979, 1980). Although designated the nucleus olfactoretinalis (NOR) by Münz and co-workers (1981, 1982), the cells appear to be part of the TN as recently characterized in goldfish (see Demski *et al.*, 1982; Demski and Northcutt, 1983; Springer, 1983; Stell
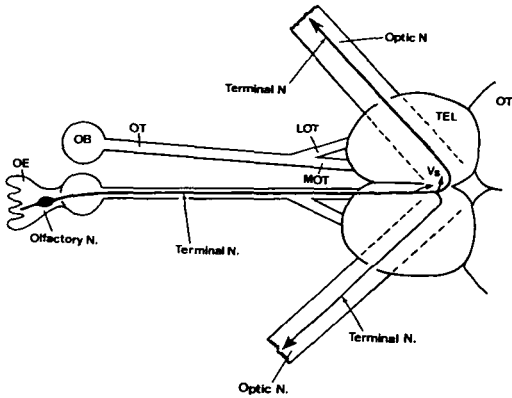
FIG. 3. Dorsal view of the olfactory system and telencephalon of goldfish (*Carassius auratus*) illustrating the distribution of the terminal nerve (TN). The pathway was traced by following the transport of horseradish peroxidase (HRP) administered to the olfactory epithelium and central processes of severed optic nerves. The stylized cell shown in the figure represents a composite of several neuronal types that contribute to the TN (see text for details). Abbreviations: lot, lateral olfactory tract; mot, medial olfactory tract; ob, olfactory bulb; oe, olfactory epithelium; ot, olfactory tract (undivided); tel, telencephalon; Vs, area ventralis telencephali pars supracommissuralis. From Demski and Northcutt (1983); reproduced from *Science* with permission.

et al., 1984). Many cells in this nucleus have processes that extend to the retina (Stell *et al.*, 1984). The heaviest projection is to the contralateral side; however, at least in goldfish, some of the cells project to the ipsilateral retina while others appear to lack the optic projection entirely (Demski and Northcutt, 1983; Stell *et al.*, 1984). It is perhaps the latter cells that are most comparable to the TN of tetrapods (see later discussions) since this particular population in goldfish sends distal processes into the olfactory epithelium and central projections to the nuclei (Vs) just dorsal to the anterior commissure (Demski and Northcutt, 1983) which, as mentioned above, contain steroid-concentrating neurons (Fig. 3). Demski and Northcutt (1983) have suggested that the TN may mediate responses to sexual pheromones produced by female goldfish. Their hypothesis is based on observations that damage to the Vs in male goldfish drastically reduces courtship (Kyle and Peter, 1982) and that the medial olfac-

tory tract (MOT), which contains the TN fibers in goldfish, must be intact for normal levels of courtship (Stacey and Kyle, 1983) as well as for mediation of sperm release triggered by electrical stimulation of the undivided olfactory tract (Fig. 4; Demski *et al.*, 1982; Demski and Dulka, 1984). Schreibman and co-workers report that the cell group is the first nucleus in which LHRH-immunoreactivity is observed during development in platyfish (Halpern-Sebold and Schreibman, 1983). They also indicate that staining for LHRH increases following hypophysectomy and that this increase can be reversed by gonadotropin injection (Schreibman *et al.*, 1983). The authors feel that cells of the nucleus transmit environmental influences to more caudal LHRH-containing centers (see below) which in turn function in the development and maintenance of reproductive systems, *i.e.*, the TN (NOR) is thought to be the initiator of changes that trigger or at least accompany sexual development. LHRH-containing fiber pathways to LHRH-reactive cells in the PON and tuberal nuclei as well as to non-reactive cell groups (*e.g.*, the habenular nucleus) are thought to be substrates for this control (see also Schreibman *et al.*, 1982).

LHRH-containing cells are also found in the PON of several species. In most cases, the cells are described as part of the nucleus preopticus periventricularis (NPP of Peter and Gill, 1975). In goldfish, the cells are situated in the ventrolateral part of the NPP where they appear to be mostly bipolar with a rostrocaudal orientation (Kah *et al.*, 1982). A similar LHRH-containing cell group has been found in platyfish (Halpern-Sebold and Schreibman, 1983), although Münz and co-workers (1981) named it nucleus preopticus basalis lateralis (Fig. 2). The cells are located adjacent to steroid-concentrating neurons and this contiguity may be a substrate for important functional interactions between the hormonal systems. As opposed to the situation for TN ganglion cells, the LHRH staining in the NPP decreases following hypophysectomy in platyfish, and the response can be reversed by gonadotropin administration (Schreibman *et al.*, 1983). This observation is consistent
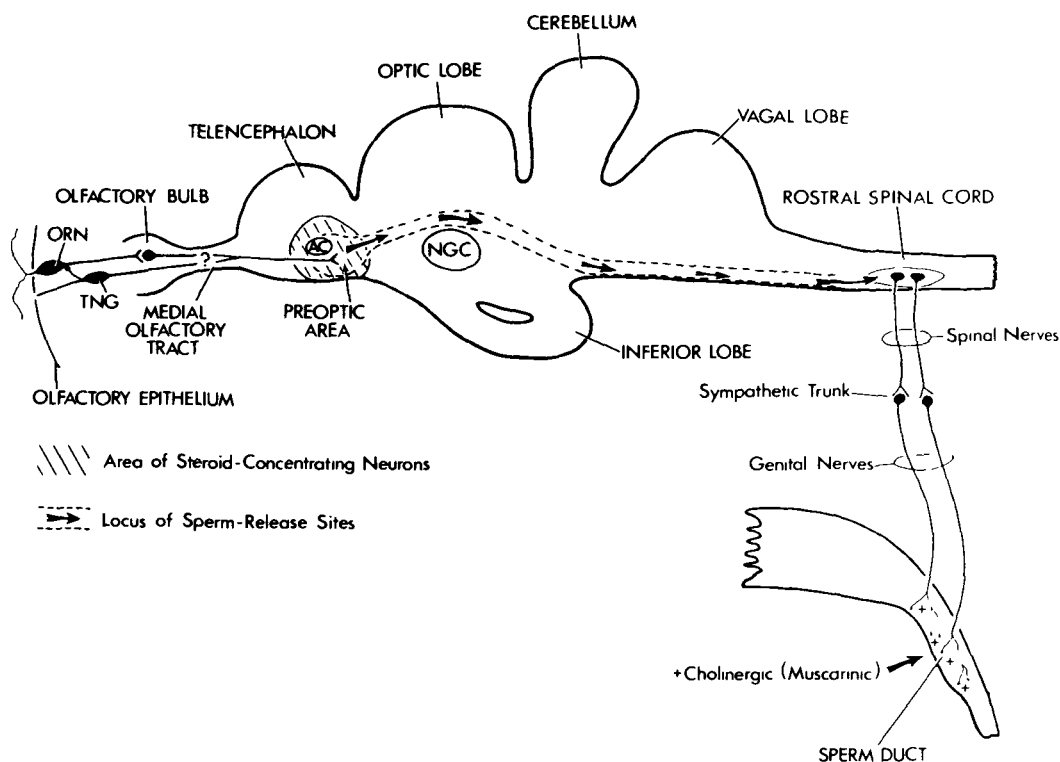
FIG. 4. Schematic sagittal section of the goldfish brain illustrating proposed pathways for chemosensory modulation of sperm release (SR). A system controlling SR extends from the preoptic area to rostral spinal cord where it leaves the CNS via spinal nerves that send fibers into the sympathetic trunks. After a probable synapse, the pathway continues via genital nerves to the sperm ducts. The neuromuscular system is cholinergic with muscarinic receptors. Sex steroid-concentrating neurons (hatching) overlap the rostral end of the system and may be involved in regulating its sensitivity to various sensory inputs. Chemosensory afferents, possibly mediating excitation triggered by a female sex pheromone, travel in the medial olfactory tract (Demski and Dulka, 1984). Terminal and/or classical olfactory fibers are the most likely critical elements in this sensory modulation of the SR pathway (see details in text). Peripheral connections of the terminal nerve ganglion cell (TNG) illustrated are hypothetical. Modified after Demski and Hornby (1982). Abbreviations: AC, anterior commissure; NGC, nucleus glomerulosus complex; ORN, olfactory receptor neuron; TNG, terminal nerve ganglion cell.

with the idea that gonadal steroids exert negative feedback on LHRH secretion via cells of the NPP. Presumably, with the pituitary removed, the stainable LHRH is decreased because of high rates of secretion in the absence of negative feedback. Fiber tracts containing LHRH extend from NPP into the tuberal region and pituitary (Münz et al., 1981; Kah et al., 1982). LHRH-immunoreactive cells have also been found in the PON of carp, *Cyprinus carpio* (Pan et al., 1979) and three-spined sticklebacks, *Gasterosteus aculeatus* (Borg et al., 1982). In the latter species some of the cells have processes that contact the ventricular sur-face and in this way are similar to lamprey preoptic LHRH-containing neurons.

A third LHRH-reactive cell group is located in the posterior part of the lateral tuberal nucleus (NLT) in platyfish (Schreibman et al., 1979; Halpern-Sebold and Schreibman, 1983) and goldfish (Kah et al., 1982). In platyfish, the nucleus reacts to hypophysectomy and gonadotropin administration in the same manner as the LHRH cells of the NPP (see above and Schreibman et al., 1983). LHRH-containing fibers extend from the NLT to the pituitary and presumably at least some of these axons originate from cells in the NLT
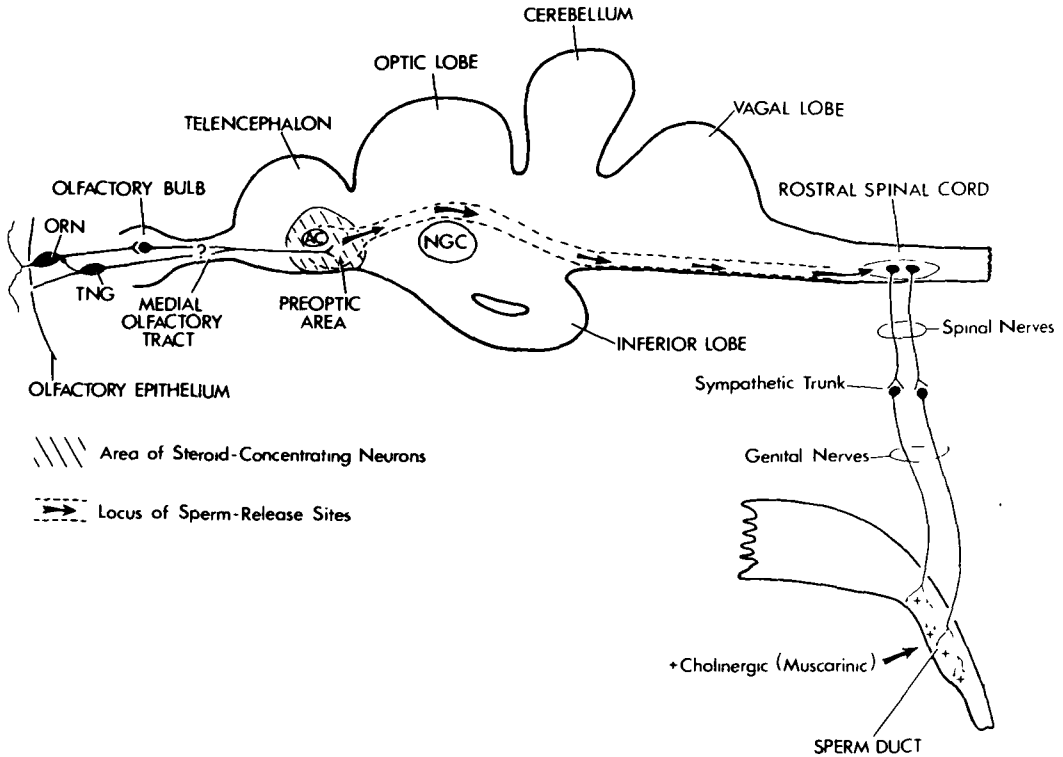
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(Schreibman et al., 1979). Whether or not some of the steroid-concentrating cells in the NLT are also LHRH-containing remains to be determined. The NLT is known to mediate steroid feedback control of gonadotropin secretion via LHRH output to the anterior pituitary (see discussions in Schreibman et al., 1979 and Peter, 1982).

A fourth group of LHRH-reactive neurons has been identified in the dorsal tegmentum of the midbrain. In platyfish and sticklebacks, the cells sit near the midline adjacent to the ventricular surface in a position between the posterior commissure and the oculomotor nucleus (Münz et al., 1981; Borg et al., 1982). In sticklebacks, LHRH-reactive cells have also been identified in periventricular portions of the thalamus (Borg et al., 1982). This location is coincident with the position of steroid-concentrating neurons in several species (see above) and suggests that the area is involved in certain, as of yet unknown, reproductive control functions. Small LHRH-immunoreactive cells have also been reported in the area dorsalis pars medialis of the telencephalon of rainbow trout, Salmo gairdneri (Goos and Murathanoglu, 1977).

LHRH-immunoreactive fibers have been identified in widespread areas of the teleost brain. Unfortunately, with the exception of the TN and certain projections to the pituitary, the exact origin of these fibers is unknown. For this reason, areas containing either reactive fibers and/or terminals are simply listed below and summarized as such in Table 1. In the telencephalon, fibers have been observed in widely distributed areas (see Figs. 1–9, Münz et al., 1981 and Figs. 1 and 2, Münz et al., 1982). Unfortunately, with the exception of some of the ventral subpallial groups, most of the nuclei of the forebrain are not labeled or otherwise indicated in any detail in the figures or text of the studies available. Thus, for purposes of this review, some of the pallial regions containing LHRH fibers as illustrated in papers by Münz et al. (1981, 1982) have been tentatively identified using the terminology of Northcutt and Braford (1980). Stained fibers appear to be in the area ventralis pars

ventralis and lateralis (Vv and Vl) in the platyfish (Schreibman et al., 1979; Münz et al., 1981), a cichlid, Cichlasoma biocellatum (Münz et al., 1982) and probably also the goldfish (Kah et al., 1982). The fibers are most likely processes of the TN cells. Reactive fibers are also present in the more posterior and supracommissural parts of the area ventralis (Vp and Vs) in at least platyfish (Münz et al., 1981). In the pallial regions, the reactive fibers are located in the medial part of the area dorsalis telencephali (Dm) in platyfish (Münz et al., 1981), cichlids (Münz et al., 1982) and goldfish (Kah et al., 1982); in the lateral part of the area dorsalis telencephali (Dl) in the platyfish (Münz et al., 1981) and cichlids (Münz et al., 1982); and in the posterior part of the area dorsalis telencephali (Dp) in platyfish (Münz et al., 1981). In the diencephalon, LHRH-containing fibers have been identified in the POA, the tuberal region, the neurohypophysis and habenula in most of the species studied (see above references), the organum vasculosum lamina terminalis (OVLT) in eels (Nozaki and Kobayashi, 1979) and nucleus diffusus lobi inferioris in goldfish (Kah et al., 1982). In the midbrain, fibers are stained in the optic lobes in goldfish (Kah et al., 1980), platyfish (Münz et al., 1981) and eels (Nozaki and Kobayashi, 1979, 1980), and the torus semicircularis in platyfish (Münz et al., 1981). In platyfish (Münz et al., 1981) and goldfish (Kah et al., 1982), scattered LHRH-reactive fibers have been identified in the cerebellum and various tegmental areas including: the reticular formation, the medial longitudinal fasciculus, the acoustico-lateral area and the nucleus of the solitary tract. In addition, Münz and co-workers (1981) suggest that LHRH-containing fibers in platyfish probably extend into the spinal cord.

Amphibians

The distribution of steroid-concentrating neurons is best documented in the African clawed frog, Xenopus laevis, in which autoradiography has been carried out using estradiol (Morrell et al., 1975b), testosterone (Kelley et al., 1975) and the non-aromatizable dihydrotestosterone (Kelley,

1980; Erulkar *et al.*, 1981). Areas containing labeled neurons include: the ventral striatum, ventral and lateral septal nuclei, nucleus accumbens, the amygdala, the POA, the ventral infundibular nucleus, the posterior thalamus, the ventral thalamus, the torus semicircularis, the dorsal tegmentum of the medulla including motor nuclei of cranial nerves IX and X and the rostral spinal cord. Androgens, in particular, were associated with vocal communication systems (motor nuclei of IX and X control the larynx and acoustic information is processed in the torus semicircularis) and motor neurons in the spinal cord involved in the androgen-dependent clasping reflex (sternoradialis and flexor carpi radialis). With the exception of the medullary and spinal motor nuclei, somewhat similar distributions of steroid-concentrating cells have been found in *Rana pipiens* (Kelley *et al.*, 1978).

LHRH-containing neurons are located in a major nuclear group beginning rostrally in the median septal area and extending caudoventrally into the anterior POA in both urodeles (*Cynops pyrrhugaster*, Kubo *et al.*, 1979; Nozaki and Kobayashi, 1979) and anurans (*Bufo arenarum*, Knigge and Pasquier, unpublished; *Rana catesbeiana*, Alpert *et al.*, 1976; Nozaki and Kobayashi, 1979; *Rana esculenta*, Goos *et al.*, 1976; *Rana pipiens*, Alpert *et al.*, 1976; and *Xenopus laevis*, Doerr-Schott and Dubois, 1976; Nozaki and Kobayashi, 1979, 1980). In at least several cases, the cell population also appears to extend rostrally into the area of the nucleus of the diagonal band (Fig. 5) and caudodorsally into the bed nucleus of the hippocampal commissure. In *Xenopus*, immunostained cells have also been reported in the olfactory bulbs, optic tectum (Nozaki and Kobayashi, 1980), the perichiasmic area and infundibulum (Doerr-Schott and Dubois, 1976; Nozaki and Kobayashi, 1980).

Fiber systems reactive to LHRH-antisera are widespread in the neuraxis. In most species studied, tracts from the area of reactive perikarya in the septum pass through the POA and end in the median eminence (see references above). The most extensive LHRH fiber distribution has been
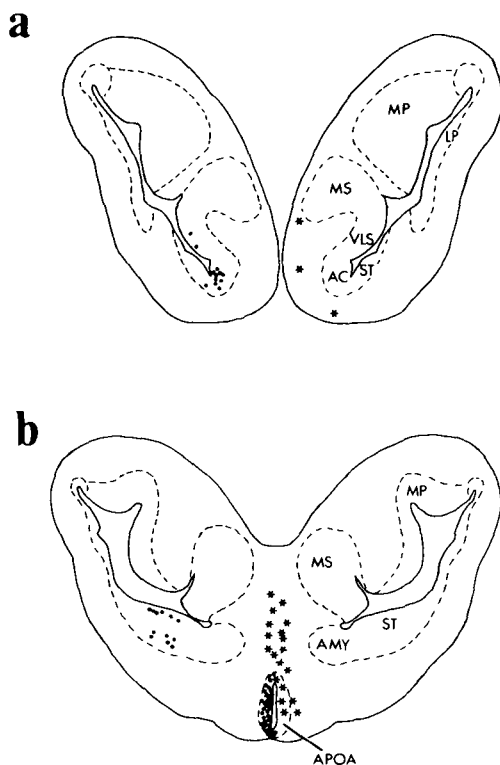
a

b

FIG. 5. Transverse sections through the anterior telencephalon (a) and preoptic area (b) of the leopard frog, *Rana pipiens* (redrawn from Kelley *et al.*, 1978). Solid dots (left side) represent every neuron autoradiographically labeled following ³H-estradiol administration in *R. pipiens* (Kelley *et al.*, 1978). Asterisks (right side) indicate the general position of LHRH-containing perikarya in *R. catesbeiana* (Alpert *et al.*, 1976) and *R. arenarum* (Knigge and Pasquier, unpublished). Immunostained cells are located primarily in the preoptic area (b) and median septal nucleus (unlabeled in b) and area of the diagonal band (unlabeled in a). Note the possible contiguity of the two hormonal systems in septal areas in rostral telencephalon (a) and definite overlap in the preoptic area (b). Brains of the *Rana* species appear to be sufficiently similar to permit these cross-species comparisons. Abbreviations: AC, anterior commissure; AMY, amygdala; APOA, anterior preoptic area; LP, lateral pallium; MP, medial pallium; MS, medial septal nucleus; ST, striatum; VLS, ventral lateral septum.

described in the South American toad, *Bufo arenarum* (K. M. Knigge and D. Pasquier, personal communications; for details contact K. M. Knigge, University of Rochester, N.Y.) In this species, there are at least four pathways emanating from the septal-POA LHRH-containing cells. An anterior

bundle runs rostroventrally into the area of the diagonal band (olfactory tubercle of Northcutt and Kicliter, 1980), giving off projections to medial septal nuclei as it passes forward. A second bundle passes along the walls of the third ventricle with apparent projections to the POA, ventral thalamus and habenula. The pathway continues caudally near the mesencephalic and fourth ventricles, distributing fibers to the central grey-toral nuclei. The system appears to eventually reach the spinal cord. The third pathway extends caudally from the septal area in a position along the lateral surface of the diencephalon. In the midbrain, fibers spread dorsally along the optic tract to enter the optic tectum where they fan out in the superficial stratum. The fourth tract is a tight bundle that follows the floor of the third ventricle to the tuberal area. Some fibers appear to end in the median eminence while others continue into the pituitary. LHRH-containing axons also project to the the olfactory bulb and habenula in newts (Kubo *et al.*, 1979).

Overlap between steroid-concentrating areas and LHRH-containing cells and fibers appears to occur in the POA of at least anurans (Fig. 5). As in fishes, this contiguity is likely to represent a substrate for steroid control of LHRH release. With regard to the TN, there is no direct evidence that it contains LHRH in amphibians, although LHRH-immunoreactive cells have been reported in the olfactory bulbs of *Xenopus* (Nozaki and Kobayashi, 1980). Its forebrain projections, however, strongly suggest that it is homologous with the TN of teleosts and, like its counterpart in fishes, interacts with systems controlling reproductive behavior and physiology. Golgi studies in frogs and salamanders demonstrate projections of the TN to both the diagonal band-median septal region and the POA (Fig. 6). Thus, the TN of amphibians appears to provide chemosensory input into areas in which either steroids and/or LHRH have been localized.

Reptiles

Among reptiles, the distribution of sex steroid-concentrating neurons has been best studied in the common green anole,

Anolis carolinensis. With few exceptions (see below), the localization patterns for labeled estradiol, testosterone and dihydrotestosterone appear to be basically similar in both sexes (Martinez-Vargas *et al.*, 1978; Morrell *et al.*, 1979). After estrogen administration, many labeled cells were found in the amygdala, nucleus of the diagonal band, bed nucleus of the stria terminalis, septum (mostly lateral), medial POA, anterior hypothalamic area, ventromedial and periventricular nuclei of the hypothalamus, torus semicircularis, and nucleus isthmi. Small numbers of labeled cells were identified in the medial, dorsal and lateral pallium, nucleus accumbens, motor nucleus of the trigeminal nerve, the raphe nuclei, reticular formation and non-motor nuclei of the rostral spinal cord. In most areas, fewer labeled cells were seen following androgen administration; exceptions include: the caudal part of the lateral pallium, lateral part of the dorsal pallium and the midbrain tegmentum. A few labeled cells were also found in the dorsal ventricular ridge. Localization of estradiol and testosterone has also been studied in both male and female garter snakes, *Thamnophis* sp. (Halpern *et al.*, 1982). Again, the distributions of concentrating-cells were similar for both sexes and estradiol generally labeled more cells. In a pattern similar to that reported for *Anolis*, concentrating-cells were identified in the amygdala including nucleus sphericus, septum, paleostriatum, retrobulbar pallium (considered dorsal pallium in Table 1), bed nucleus of stria terminalis, POA, anterior periventricular, ventromedial and arcuate nuclei of the hypothalamus, central grey and other areas of the midbrain and medullary tegmentum. A few scattered cells were also found in the optic tectum and inferior colliculus (torus semicircularis). In red-eared turtles, *Pseudemys scripta elegans*, the pattern of [^{3}H]estradiol has been studied in both males and females (Kim *et al.*, 1981*a*). Estrogen target neurons were found in cortical paraolfactory areas, the amygdala, dorsal ventricular ridge, piriform cortex, nucleus of the diagonal band, bed nucleus of stria terminalis, nucleus accumbens, septum (medial and lateral), POA, periventricular
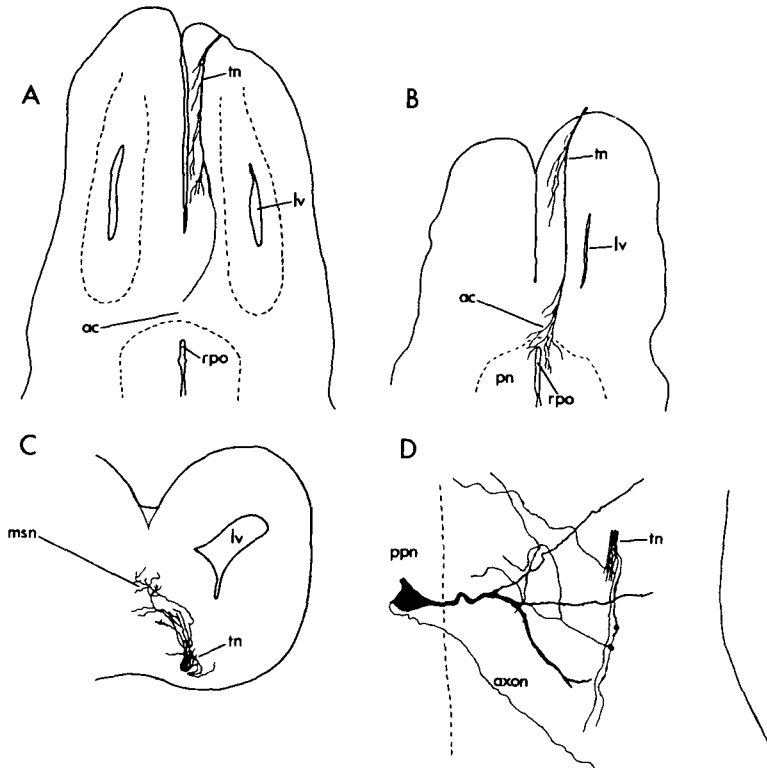
FIG. 6. Central projections of the terminal nerve (tn) in amphibians as determined from Golgi preparations. A. Horizontal section of the brain of an adult cricket frog, *Acris gryllus.* B. Horizontal section of the brain of a larval tree frog, *Hyla* sp. C. Transverse section of the brain of a larval *Rana* sp. D. Horizontal section of the brain of the mud puppy, *Necturus maculosus* illustrating a neuron in the right preoptic area with its dendrites overlapping axons from the terminal nerve. In this figure the dashed line indicates the lateral border of the cellular zone of the preoptic nucleus and the solid line represents the lateral border of the entire preoptic area. Dashed lines in A and B indicate the borders of cellular areas. Note the distribution of terminal nerve fibers to the medial septal areas in A–C and preoptic region in B and D. A, B and D were redrawn from McKibben (1911); C was traced from Herrick (1909). Abbreviations: ac, anterior commissure; lv, lateral ventricle; msn, median septal nucleus; pn, preoptic nucleus; ppn, cellular zone of preoptic area; rpo, preoptic recess; tn, terminal nerve.

areas in anterior and medial hypothalamus (ventromedial area), infundibulum, thalamus adjacent to nucleus rotundus, torus semicircularis and central grey of the midbrain, nucleus isthmi and reticular formation. Thus, the distribution of the sex steroid-concentrating cells among reptiles is strikingly similar and comparisons with data from birds and mammals (see below) indicate that this generalized reptilian distribution may represent a basic amniote pattern.

Information on LHRH systems in reptiles is sparce. Nozaki and Kobayashi (1979) have carried out immunocytochemical studies in a variety of snakes and lizards but have had little success in most species. Their best results were obtained in the snake, *Elaphe climacophora.* Stained perikarya are clearly illustrated in the median septal nucleus and adjacent medial POA. A few positive cells were also observed in the bed nucleus of the hippocampal commissure. The majority of stained fibers leaving these areas pass ventrocaudally into the median eminence. In a later abstract (1980), the authors report that "In most species of reptiles and amphibians, intra- and extra-hypothalamic LH-RH pathways that proceed toward (1) the median eminence, (2) the olfactory bulb, (3) the optic tectum and (4) the lower brain stem were
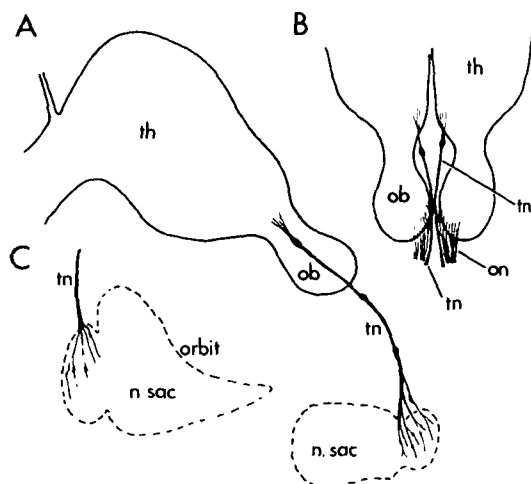
FIG. 7. Distribution of the terminal nerve (tn) in turtle embryos (Emydidae). In A the terminal nerve is projected on a parasagittal plane showing the medial hemispheric wall. B illustrates the projection of the terminal and olfactory nerves in a dorsal view of both hemispheres. C is a transverse section of the nasal sac indicating the peripheral distribution of the terminal nerve to what is believed to be the vomeronasal area. A–C were redrawn after Johnston (1913). Abbreviations: n. sac, nasal sac; ob, olfactory bulb; on, olfactory nerve; th, telencephalic hemisphere; tn, terminal nerve.

demonstrated." The limited information available on reptiles suggests a basic similarity to the patterns observed in amphibians, including an overlap of steroid-concentrating cells and LHRH-containing neurons in the septal area.

There are as of yet no reports of LHRH staining in the TN of reptiles; however, the observation of LHRH-immunoreactive fibers running toward the olfactory bulb in certain species (see above) may indeed represent fibers of the TN. The classical anatomy of the TN of reptiles (Johnston, 1913; Larsell, 1919) suggests that it is similar to the TN of amphibians and mammals (see below). Ganglion cells located at several positions between the olfactory bulb and the nasal sac send peripheral processes to the olfactory epithelium and central processes into median hemispheric areas (Fig. 7).

Birds

Steroid hormone-concentrating neurons have been studied in the ring dove,

Streptopelia risoris (Martinez-Vargas *et al.*, 1975, 1976), domestic fowl (Wood-Gush *et al.*, 1977; Barfield *et al.*, 1978) and two passerines, the zebra finch, *Poephila guttata* (Arnold *et al.*, 1976) and the chaffinch, *Fingillia coelebs* (Zigmond *et al.*, 1980). In cases where labeled hormones have been given to both sexes, there do not appear to be significant differences in uptake patterns (Martinez-Vargas *et al.*, 1975; Wood-Gush *et al.*, 1977). Differences between patterns for androgens and estrogens have been reported in the one study in which both hormone types were given to animals of the same sex (Wood-Gush *et al.*, 1977). The following areas, listed with their most probable mammalian counterparts in parentheses, contain many well-labeled cells in all of the species studied: the nucleus taeniae and adjacent archistriatum (amygdala), the nucleus interstitialis (bed nucleus of stria terminalis), periventricular and medial hypothalamic nuclei (VMN and premammillary nuclei), infundibular-tuberal nuclei (arcuate nucleus), nucleus intercollicularis of the midbrain (central grey of mammals, part of torus semicircularis of other vertebrates). In several species, cells were labeled in the thalamus, areas near the isthmo-optic nucleus and the nuclei of cranial nerves III and V. Other positive cells were found in scattered regions of the pontomedullary tegmentum, the area of the nucleus accumbens and the bed nucleus of the diagonal band, the neo-, hyper- and paleostriatum, paraolfactory lobes and, at least in the ring dove, the olfactory tubercle, hypothalamus and spinal nuclei (non-motor). In the two song birds (zebra and chaffinch), several structures involved in vocalization demonstrated heavy labeling with testosterone. These include: the magnocellular nucleus of the anterior neostriatum, the caudal part of the ventral hyperstriatum, and sonic motor cells in the nucleus of the hypoglossal nerve. It appears that vocal control pathways of at least some song birds are especially prone to modulation via gonadal steroids. This is not surprising since singing in these species is androgen dependent (see details in Arnold *et al.*, 1976).

LHRH-immunoreactive material has

been found in preoptic neurons in a variety of birds. The cells appear to send ventro-caudally directed fibers toward the median eminence (McNeill *et al.*, 1976; Bons *et al.*, 1978; Hoffman *et al.*, 1978; Oksche, 1978; Józsa and Mess, 1982; Sterling and Sharp, 1982). In most of the species, many reactive perikarya have also been observed in septal nuclei. In chickens, Sterling and Sharp (1982) report more cells in lateral septal areas while Józsa and Mess (1982) and Hoffman and co-workers (1978) observed a heavier staining in medial septal nuclei. Observations in pheasants are consistent with the stronger medial distribution (Hoffman *et al.*, 1978). Reactive cells have also been reported in the tuberal-infundibular nuclei (McNeill *et al.*, 1976; Hoffman *et al.*, 1978); however, these results have been questioned by Sterling and Sharp (1982) who, like several other investigators, failed to locate LHRH cells in this area. They point out that the antisera used in producing the initial positive results cross-reacts with ACTH, thus making the identification ambiguous. Other LHRH-immunoreactive perikarya have been located near the OVLT in chickens (Józsa and Mess, 1982), and in the olfactory bulb (Hoffman *et al.*, 1978; Józsa and Mess, 1982) and the lobus paraolfactorius (Hoffman *et al.*, 1978) in both chickens and pheasants.

LHRH-immunoreactive fibers have been found in the POA, periventricular hypothalamic areas, infundibular-tuberal nuclei and the median eminence in all species studied (see above references and Nozaki and Kobayashi, 1979). The fibers are thought to originate from both POA and septal cells. In at least the chicken, many fibers project from the septal-POA nuclei forward to the bed nucleus of the pallial commissure and the olfactory bulbs and caudally to the OVLT (Józsa and Mess, 1982; Sterling and Sharp, 1982), the nuclei of the tectal commissure, the subcommissural organ and via the stria medullaris to the habenula, central grey and interpeduncular area (Józsa and Mess, 1982).

Close approximation of steroid-concentrating cells and LHRH pathways in birds appears to occur in several areas. Neurons related to both systems are found in the septum where as in reptiles and mammals there appears to be a tendency for steroid-concentrating cells to be lateral to the LHRH-containing neurons (see text and Table 1). Other areas of overlap between the systems occur in the midbrain tegmentum and periventricular portions of the POA and hypothalamus.

A description of the TN in birds could not be found, although statements have been made that the structure is present in all vertebrate classes (Ariëns Kappers *et al.*, 1936). Its absence in birds would not be surprising since chemosensory systems have been greatly reduced in this group, *e.g.*, the vomeronasal system appears to be lost entirely (Northcutt, 1981). The observation of LHRH-reactive perikarya and fibers in the olfactory bulb and rostrobasal telencephalon does, however, suggest that at least remnants of the TN may still be present.

Mammals

The distribution of steroid-concentrating neurons in mammals has been thoroughly reviewed (Stumpf, 1970*b*; Morrell *et al.*, 1975*a*; Morrell and Pfaff, 1978; Stumpf and Sar, 1978; Pfaff, 1980) and all authors have stressed the similarities in basic patterns among different species. For this reason, neither the details of the patterns in individual groups nor differences among distributions for labeled estradiol, testosterone and dihydrotestosterone have been included in this paper. Instead, a list of structures which contain the sex steroid-concentrating cells in mammals as a group was compiled and incorporated into Table 1. With few exceptions (see below), the distributions of labeled cells appear to be similar in both sexes. In addition to the reviews listed above, the following references were consulted: rodents (Pfaff and Kiner, 1973; Sar and Stumpf, 1975*a*, *b*; Stumpf *et al.*, 1975; Krieger *et al.*, 1976; Warembourg, 1977*a*, *b*; Sheridan, 1978; Breedlove and Arnold, 1980; Morrell *et al.*, 1982), carnivores (Morrell *et al.*, 1977), and primates (Keefer and Stumpf, 1975*a*, *b*; Pfaff *et al.*, 1976; Warembourg, 1977*c*; Sheridan and Weaker, 1982; Sheridan *et al.*, 1982).
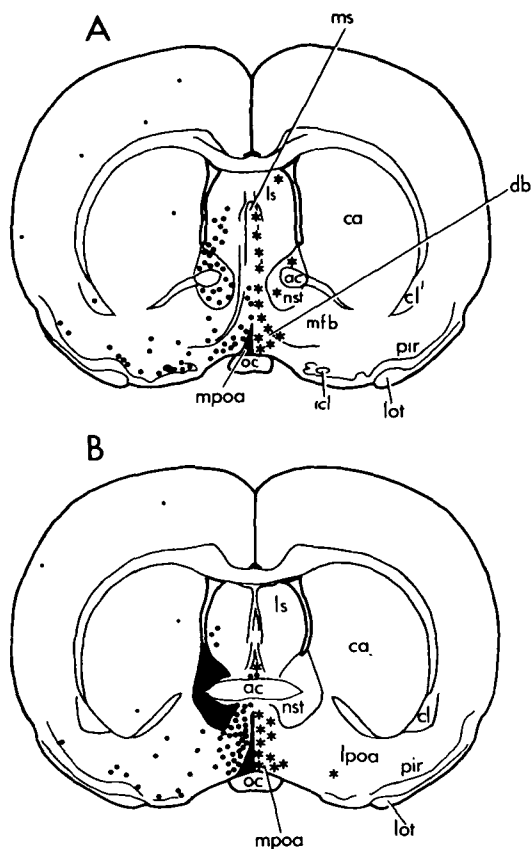
FIG. 8. Transverse sections through the septal and preoptic areas of the rat brain; A is rostral to B (redrawn from Pfaff and Keiner, 1973). Black dots (left side) represent estrogen-concentrating neurons. Solid-black areas indicate zones where the dots would merge (Pfaff and Keiner, 1973). Asterisks mark brain regions with LHRH-containing cell bodies (replotted from Witkin *et al.*, 1982). Note the relative position of the steroid-concentrating cells in the lateral septal area and the LHRH-containing neurons in the medial septal region. The systems appear to be at least adjacent in the mid-lateral zone of the septum. More striking contiguity between the two cell types is apparent in the bed nucleus of the stria terminalis (nst) and medial preoptic area (mpoa) where the general distributions for the two neurohormonal systems overlap. Abbreviations: ac, anterior commissure; ca, caudate nucleus; cl, claustrum; db, diagonal band of Broca; icl, Island of Calleja; lot, lateral olfactory tract; lpoa, lateral preoptic area; ls, lateral septum; mfb, medial forebrain bundle; mpoa, medial preoptic area; ms, medial septum; nst, bed nucleus of the stria terminalis; oc, optic chiasm; pir, prepiriform cortex.

In summary, areas in mammals with high densities of steroid-concentrating neurons include (Fig. 8): the olfactory tubercle, the nucleus accumbens, the bed nuclei of the

diagonal band and stria terminalis, the lateral septum, the amygdala, the POA-anterior hypothalamus, the infundibular, tuberal, ventromedial and premammillary nuclei of the hypothalamus, the habenula, portions of the thalamus, the central grey of the midbrain, scattered areas in the tegmentum of the lower brainstem and both nonmotor and motor nuclei of the spinal cord, *e.g.*, the androgen-concentrating nucleus that innervates the sexually dimorphic bulbocavernosus muscle in rats (Breedlove and Arnold, 1980, 1981).

There have been many recent investigations on the distributions of LHRH-immunoreactive nerve cells and fibers in the brain of various mammals ranging from rodents to primates, including humans. Comparisons among species have been made in several comprehensive reviews (Flerko *et al.*, 1978; Hoffman *et al.*, 1978; Silverman and Zimmerman, 1978; Barry, 1979; Silverman *et al.*, 1979). For purposes of this discussion, the information in these reviews was supplemented by data from the following more recent studies (see below and Table 1): guinea pig (Silverman and Krey, 1978; Schwanzel-Fukuda and Silverman, 1980), hamster (Jennes and Stumpf, 1980; Phillips *et al.*, 1980, 1982), rat (Liposits and Sétáló, 1980; Merchenthaler *et al.*, 1980; Dluzen and Ramirez, 1981; Bennett-Clarke and Joseph, 1982; Liposits *et al.*, 1982; Witken *et al.*, 1982), and primates (Marshall and Goldsmith, 1980; Silverman *et al.*, 1982).

In most cases, LHRH-immunoreactive cells have been located in a more or less continuous distribution extending from the medial septal area through the suprachiasmatic region of the medial POA into periventricular areas of the hypothalamus (Fig. 8). In some studies, more anterior reactive cells have been identified in one or more of the following telencephalic areas: nucleus of the diagonal band, bed nucleus of the stria terminalis, olfactory tubercle, anterior olfactory nucleus, prepiriform cortex, main and accessory olfactory bulbs, and TN (see below). In many species, cells have also been located in infundibular and tuberal regions such as the arcuate nucleus; however, the specificity of staining in this

area has been questioned (see Flerko *et al.*, 1978; Barry, 1979).

In various species, LHRH-immunoreactive fibers have been described which include projections from cells in the POA-septal region to: 1) OVLT and median eminence, 2) mammillary nuclei and ventral tegmentum via the hypothalamus and 3) ventral tegmentum via stria medullaris, habenula and fasciculus retroflexus. Immunoreactive fibers have also been associated with the amygdala, supraoptic area, lateral hypothalamus, central grey, superior colliculus, brainstem reticular formation and TN. Reactive fibers have also been observed in the TN (see below).

Areas of apparent overlap between sex steroid-concentrating neurons and LHRH-immunoreactive systems include: the POA, medial-basal hypothalamus (tuberal-infundibular area), the olfactory tubercle, bed nucleus of stria terminalis, nucleus of the diagonal band, nucleus accumbens and nucleus triangularis septi (Fig. 8). In the septum, the two types of cells appear to be adjacent with the steroid-concentrating neurons lateral to the LHRH-containing perikarya (Fig. 8). Regions which contain steroid-concentrating cells and receive LHRH-immunoreactive fibers and/or terminals include: the areas described above, the amygdala, the ventromedial and premammillary nuclei of the hypothalamus, the habenula, and central grey and other areas in the brainstem tegmentum. Although less well-documented, anatomical overlap between the two neurohormonal systems may also occur in the olfactory bulb, cingulate cortex, subfornical organ and optic tectum.

The general peripheral and central distribution of the TN in a variety of species including humans has been known for some time (Johnston, 1913; Larsell, 1918; and others). Recently the system has been mapped in detail in fetal and adult guinea pigs using LHRH immunocytochemistry (Schwanzel-Fukuda and Silverman, 1980). In this species, the nerve consists of a plexus of ganglion cells and fibers which extends from the nasal epithelium along the ventral surface of the olfactory bulbs and base of the telencephalon and enters the septal
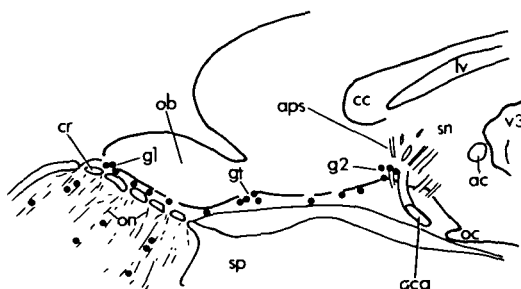

FIG. 9. Distribution of the terminal nerve in fetal guinea pigs plotted on a sagittal section through the brain, basal cranium and nasal apparatus. Solid dots indicate perikarya of terminal nerve ganglion cells immunocytochemically stained for LHRH. The reactive cells are located within a plexus of LHRH-containing fibers extending from the olfactory epithelium along the ventral surface of the brain to the area of penetration of the anterior cerebral artery. Some of the cells are grouped into three distinct ganglia (g1, g2 and gt). Many non-reactive cells were also scattered through the system (not illustrated). Redrawn from Schwanzel-Fukuda and Silverman (1980). Abbreviations: ac, anterior commissure; aca, anterior cerebral artery; aps, anterior perforated substance; cc, corpus callosum; cr, cribiform plate of the ethmoid bone; g1, terminal nerve ganglion on ventromedial aspect of the rostral olfactory bulb; g2, terminal nerve ganglion in proximity to branches of the anterior cerebral artery; gt, terminal nerve ganglion ("ganglion terminale") just caudal to the olfactory bulb; lv, lateral ventricle; ob, olfactory bulb; oc, optic chiasm; on, olfactory nerve; sn, septal nuclei; sp, sphenoid bone; v3, third ventricle.

region along with penetrating branches of the anterior cerebral artery. LHRH immunoreactive fibers appear to interconnect at least three descrete ganglia located along the TN pathway (Fig. 9). Similar LHRH-containing TN pathways have also been reported in the hamster (Jennes and Stumpf, 1980), rat (Witken *et al.*, 1982) and rhesus and pigtailed macaques (Silverman *et al.*, 1982). The TN of mammals like the similar structure in teleosts (see above) may function by permitting certain chemical stimuli, most likely pheromones, to influence reproductive activity via LHRH modulation of central regions such as the septal-POA (see discussions in Schwanzel-Fukuda and Silverman, 1980, and Demski and Northcutt, 1983).

CONCLUSIONS

As a means for synthesizing the wealth of data considered in this paper, areas of

PRIMARY SENSORY	SECONDARY SENSORY SENSORIMOTOR	MOTIVATIONAL- AUTONOMIC	MOTOR
●OLFACTORY NERVE	✱OLFACTORY TUBERCLE	✱CINGULATE CORTEX	▲VOCAL CONTROL AREAS
●OLFACTORY BULB	▲STRIATUM	✱HIPPOCAMPUS	▲MOTOR CRANIAL NUCLEI
●ACC. OLFACTORY BULB	✱THALAMUS	✱AMYGDALA	▲SPINAL NUCLEI:
●RETINA	✱TECTUM	✱SEPTUM	(BULBOCAVERNOSUS)
	✱TORUS SEMICIRCULARIS	✱HABENULA	(FLEXOR CARPI RADIALIS)
	✱CENTRAL GREY	✱PREOPTIC AREA	(STERNORADIALIS)
	✱CEREBELLUM	✱HYPOTHALAMUS	
	▲LAT. VESTIBULAR NUCLEUS		
	▲SPINAL N. OF V		
	▲N. SOLITARY TR.		
	▲SPINAL CORD-DORSAL HORN		

●LHRH PATHWAYS
▲STEROID-CONCENTRATING CELLS
✱BOTH●+▲

FIG. 10.   Distribution of LHRH-containing neural pathways and sex steroid-concentrating cells with respect to function. Anatomical data for all vertebrate groups discussed in the text (see Table 1) were pooled in compiling this figure. See Conclusions for details.

the brain containing either sex steroid-concentrating neurons and/or LHRH-containing cells or fibers were placed into the following functional categories: 1) primary sensory, 2) secondary sensory-sensorimotor, 3) motivational-autonomic and 4) motor (Fig. 10). Despite some overlap, the groupings approximate major functional categories commonly used by neuroethologists (see Ewert, 1980; Guthrie, 1980). The results suggest several functional-anatomical correlations which are likely to reflect important evolutionary patterns.

Androgenic control of motor pathways for male sexual behavior appears to have evolved independently at least several times. The statement is based on identification of steroid-concentrating cells in several nonhomologous systems mediating such diverse responses as singing in birds, clasping in frogs and calling in toadfish. The hormone-binding cells are present in functional mechanisms ranging from telencephalic and midbrain sensorimotor integrating centers to primary motor nuclei (see examples in text). In the latter case,

Erulkar and co-workers (1981) have demonstrated androgen-specific membrane changes in motor neurons controlling clasping in Xenopus. The changes result in increased motor cell activity in response to artificial stimulation likely to represent the "normal" tactile input that occurs during spawning. It is reasonable to assume that androgens may exert similar effects in other steroid-concentrating motor systems and that certain motor neurons may have a propensity for development of androgen receptor systems, modifications which are likely to be adaptive in cells involved in male-specific reproductive responses. Steroid-concentrating neurons probably also evolved as substrates for sex-hormone modulation of LHRH-containing brain circuits mediating gonadal development and sexual behavior (see below). Indeed, this may have first occurred in the POA of early agnathans. The hypothesis is based on the presence of estrogen-concentrating cells and LHRH-containing perikarya in the lamprey POA and the assumption that this overlapping representation is a primitive feature. The fact that similar systems

are found in teleosts and a variety of tetrapods supports the assumption.

In the early vertebrates, LHRH was probably secreted directly into the systemic circulation via neurohaemal organs such as the neurohypophysis and OVLT. The hypothesis follows from observations that LHRH-reactive terminals are present in these organs in a variety of vertebrates including lampreys. Secretion of the peptide directly into the CSF with transport within the ventricular system and eventually into the venous system may also be a primitive mechanism since LHRH-containing CSF-contacting neurons are especially characteristic of lampreys (Crim *et al.*, 1979*a*). Presumably, these "primitive" systems could have mediated LHRH modulation of anterior pituitary function as well as activity in any brain-behavior circuits with receptors for the peptide. Synchronization of sexual behavior with gonadal development was probably regulated by steroid modulation of LHRH effects through changes in the activity of steroid-concentrating neurons.

Teleosts, amphibians and amniotes have well-developed systems for local delivery of LHRH for controlling gonadotropin secretion (see review by Barry, 1979). Teleosts utilize direct innervation of the gonadotrops via LHRH-containing fibers (Schreibman *et al.*, 1979) while LHRH reaches the anterior pituitary via portal vessels of the median eminence in tetrapods (see Table 1). It appears that selective pressure for a specific control of gonadal function has thus resulted in at least two elaborations of the "primitive" pattern of secretion of LHRH into the systemic circulation. Teleosts, amphibians and other tetrapods also have additional LHRH-containing neural pathways, at least some of which appear to be homologous in the groups studied; none of the pathways have been observed in agnathans (see below and Fig. 10). These observations suggest that elaborate intracerebral systems involving LHRH as a neurotransmitter or modulator may have developed in the early gnathostome ancestors of the bony fishes and tetrapods. Functional studies in living species indicate that many of the pathways prob-

ably evolved as regulatory mechanisms controlling sexual behavior (see below and text for details). Some of the pathways may have developed by modification of "primitive" systems that were already responsive to LHRH secreted directly into the systemic circulation. The "new" adaptation would thus have been direct LHRH innervation of existing LHRH-sensitive systems. This could possibly have occurred by sprouting of collaterals from a major pool of LHRH-containing perikarya located in the POA-medial septal region where many such cells are found in all the living vertebrates that have been studied (see Table 1 and text). In summary, the gnathostomes appear to have evolved direct LHRH delivery systems to control both gonadal function and reproductive behavior. These modifications undoubtedly permitted a more efficient utilization of LHRH. Further studies in a variety of species including hagfish, elasmobranchs and primitive actinopterygians are necessary to test the hypothesis.

In gnathostomes, LHRH-containing systems are present in both primary and higher order sensory pathways (Fig. 10) and thereby probably directly influence the processing of sensory information relevant to sexual behavior. The peptide is also present in "sensorimotor integrative" regions where it may modulate the expression of reproductive responses, perhaps in association with the effects of gonadal hormones expressed via sex steroid-concentrating neurons closely associated with the LHRH pathways (Fig. 10). Brain areas involved in controlling sexual motivation and/or autonomic components of reproductive behavior (see examples in Kelley and Pfaff, 1978) also appear to be sites for interactions between the two hormonal systems (Fig. 10). Many such loci are located in limbic circuits which include both hypothalamic and epithalamic connections with the midbrain. These neuronal systems are most likely involved in sexual arousal and the mediation of appetitive behavior.

With few exceptions, (see below) the distribution of LHRH-containing and steroid-concentrating areas in the brains of amniotes is similar to that found in amphib-

ians (see Table 1 and text). One of the more obvious differences between the two groups is that limbic areas in amniotes seem to have an increased number of both hormone-specific elements (see Table 1 and text). This difference is not surprising since these regions become highly developed in reptiles, birds and mammals (see Northcutt, 1981) and, in at least certain cases, control both gonadotropin secretion and sexual behavior (Beltramino and Taleisnik, 1978; Parvizi and Ellendorff, 1980; Lehman and Winans, 1982).

With the exclusion of androgen-concentrating cells in vocal control areas of the neo- and paleostriatum of birds, neocortical regions of mammals and their probable homologs in reptiles and birds appear to lack well-developed substrates for either sex-steroid binding or LHRH modulation of neural activity. However, the strong representation of the two systems in the POA-septal region characteristic of fishes and amphibians is retained in the amniotes as is the basic intracerebral distribution of the LHRH-containing TN (see text). The latter examples underline the apparent conservative evolution of areas of overlap of LHRH-containing perikarya and fibers and sex steroid-concentrating neurons and suggest that basal forebrain systems sensitive to these hormones evolved as "necessary" components of circuits mediating highly adaptative reproductive responses.

REFERENCES

Alpert, L. C., J. R. Brawer, I. M. D. Jackson, and S. Reichlin. 1976. Localization of LHRH in neurons in frog brain (*Rana pipiens* and *Rana catesbeiana*). Endocrinology 98:910–921.

Ariëns Kappers, C. U., G. C. Huber, and E. C. Crosby. 1936. *The comparative anatomy of the nervous system of vertebrates including man.* Reprinted by Hafner, New York, 1965.

Arnold, A. P., F. Nottebohm, and D. W. Pfaff. 1976. Hormone concentrating cells in vocal control and other areas of the brain of the zebra finch (*Poephila guttata*). J. Comp. Neurol. 165:487–512.

Barfield, R. J., G. Ronay, and D. W. Pfaff. 1978. Autoradiographic localization of androgen-concentrating cells in the brain of the male domestic fowl. Neuroendocrinology 26:297–311.

Barry, J. 1979. Immunohistochemistry of luteinizing hormone-releasing hormone-producing neurons of the vertebrates. Int. Rev. Cytol. 60:179–221.

Beltramino, C. and S. Taleisnik. 1978. Facilitatory and inhibitory effects of electrochemical stimulation of the amygdala on the release of luteinizing hormone. Brain Res. 144:95–107.

Bennett-Clarke, C. and S. A. Joseph. 1982. Immunocytochemical distribution of LHRH neurons and processes in the rat: Hypothalamic and extrahypothalamic locations. Cell Tiss. Res. 221:493–504.

Bons, N., B. Kerdelhué, and I. Assenmacher. 1978. Immunocytochemical identification of an LHRH-producing system originating in the preoptic nucleus of the duck. Cell Tiss. Res. 188:99–106.

Borg, B., H. J. Th. Goos, and M. Terlou. 1982. LHRH-immunoreactive cells in the brain of the three-spined stickleback, *Gasterosteus aculeatus* L. (Gasterosteidae). Cell Tiss. Res. 226:695–699.

Breedlove, S. M. and A. P. Arnold. 1980. Hormone accumulation in a sexually dimorphic motor nucleus of the rat spinal cord. Science 210:564–566.

Breedlove, S. M. and A. P. Arnold. 1981. Sexually dimorphic motor nucleus in the rat lumbar spinal cord: Response to adult hormone manipulation, absence in androgen-insensitive rats. Brain Res. 225:297–307.

Crim, J. W., A. Urano, and A. Gorbman. 1979a. Immunocytochemical studies of luteinizing hormone-releasing hormone in brains of agnathan fishes I. Comparisons of adult Pacific lamprey (*Entosphenus tridentata*) and the Pacific hagfish (*Eptatretus stouti*). Gen. Comp. Endocrinol. 37:294–305.

Crim, J. W., A. Urano, and A. Gorbman. 1979b. Immunocytochemical studies of luteinizing hormone-releasing hormone in brains of agnathan fishes II. Patterns of immunoreactivity in larval and maturing western brook lamprey (*Lampetra richardsoni*). Gen. Comp. Endocrinol. 38:290–299.

Davis, R. E., J. I. Morrell, and D. W. Pfaff. 1977. Autoradiographic localization of sex steroid-concentrating cells in the brain of the teleost *Macropodus opercularis* (Osteichthyes: Belontiidae). Gen. Comp. Endocrinol. 33:496–505.

Demski, L. S. 1978. Neuroanatomical substrates of reproductive behavior in male sunfish (genus *Lepomis*). Ann. Biol. Anim. Bioch. Biophy. 18:831–836.

Demski, L. S. 1981. Neural mechanisms of sound production in fishes. *In* R. R. Fay, A. N. Popper, and W. N. Tavolga (eds.), *Hearing and sound com-*

munication in fishes, pp. 427–445. Springer-Verlag, New York.

Demski, L. S. and J. G. Dulka. 1984. Functional-anatomical studies on sperm release evoked by electrical stimulation of the olfactory tract in goldfish. Brain Res. 291:241–247.

Demski, L. S., J. G. Dulka, and R. G. Northcutt. 1982. Chemosensory control of spawning mechanisms in goldfish. Neurosci. Abstr. 8:611.

Demski, L. S. and J. W. Gerald. 1974. Sound production and other behavioral effects of midbrain stimulation in free-swimming toadfish. Brain Behav. Evol. 9:41–59.

Demski, L. S., J. W. Gerald, and A. N. Popper. 1973. Central and peripheral mechanisms of teleost sound production. Amer. Zool. 13:1141–1167.

Demski, L. S. and P. J. Hornby. 1982. Hormonal control of fish reproductive behavior: Brain-gonadal steroid interactions. Can. J. Fish. Aquat. Sci. 39: 36–47.

Demski, L. S. and R. G. Northcutt. 1983. The terminal nerve: A new chemosensory system in vertebrates? Science 220:435–437.

Dluzen, D. E. and V. D. Ramirez. 1981. Presence and localization of immunoreactive luteinizing hormone-releasing hormone (LHRH) within the olfactory bulbs of adult male and female rats. Peptides 2:493–496.

Doerr-Schott, J. and M. P. Dubois. 1976. LHRH-like system in the brain of *Xenopus laevis* Daud: Immunohistochemical identification. Cell Tiss. Res. 172: 477–486.

Erulkar, S. D., D. B. Kelley, M. E. Jurman, F. P. Zemlan, G. T. Schneider, and N. R. Krieger. 1981. Modulation of the neural control of the clasp reflex in male *Xenopus laevis* by androgens: A multi-disciplinary study. Proc. Natl. Acad. Sci. 78:5876–5880.

Ewert, J.-P. 1980. *Neuroethology*. Springer-Verlag, New York.

Fine, M. L. 1979. Sounds evoked by brain stimulation in the oyster toadfish *Opsanus tau* L. Exp. Brain Res. 35:197–212.

Fine, M. L., D. A. Keefer, and G. R. Leichnetz. 1982. Testosterone uptake in the brainstem of a sound-producing fish. Science 215:1265–1267.

Flerko, B., G. Sétáló, S. Vigh, A. Arimura, and A. V. Schally. 1978. The luteinizing hormone-releasing hormone (LH-RH) neuron system in the rat and rabbit. *In* D. E. Scott, G. P. Kozlowski, and A. Weindl (eds.), *Brain-endocrine interaction III. Neural hormones and reproduction*, pp. 108–116. Karger, Basel.

Goos, H. J. Th., P. J. M. Ligtenberg, and P. G. W. J. van Oordt. 1976. Immunofluorescence studies on gonadotropin releasing hormone (GRH) in the fore-brain and the neurohypophysis of the green frog, *Rana esculenta* L. Cell Tiss. Res. 168: 325–333.

Goos, H. J. Th. and O. Murathanoglu. 1977. Localization of gonadotropin releasing hormone (GRH) in the forebrain and neurohypophysis of the trout (*Salmo gairdneri*). Cell Tiss. Res. 181:163–168.

Guthrie, D. M. 1980. *Neuroethology: An introduction*. John Wiley and Sons, New York.

Halpern, M., J. I. Morrell, and D. W. Pfaff. 1982. Cellular [³H]estradiol and [³H]testosterone localization in the brains of garter snakes: An autoradiographic study. Gen. Comp. Endocrinol. 46: 211–224.

Halpern-Sebold, L. R. and M. P. Schreibman. 1983. Ontogeny of centers containing luteinizing hormone-releasing hormone in the brain of platyfish (*Xiphophorus maculatus*) as determined by immunocytochemistry. Cell Tiss. Res. 229:75–84.

Herrick, C. J. 1909. The nervus terminalis (nerve of Pinkus) in the frog. J. Comp. Neurol. Psychol. 19:175–190.

Hoffman, G. E., V. Melnyk, T. Hayes, C. Bennett-Clarke, and E. Fowler. 1978. Immunocytology of LHRH neurons. *In* D. E. Scott, G. P. Kozlowski, and A. Weindl (eds.), *Brain-endocrine interaction III. Neural hormones and reproduction*, pp. 67–82. Karger, Basel.

Jennes, L. and W. E. Stumpf. 1980. LHRH-systems in the brain of the golden hamster. Cell Tiss. Res. 209:239–256.

Johnston, J. B. 1913. Nervus terminalis in reptiles and mammals. J. Comp. Neurol. 23:97–120.

Józsa, R. and B. Mess. 1982. Immunohistochemical localization of the luteinizing hormone releasing hormone (LHRH)-containing structures in the central nervous system of the domestic fowl. Cell Tiss. Res. 227:451–458.

Kah, O., P. Chambolle, P. Dubourg, and M. P. Dubois. 1982. Distribution of immunoreactive LHRH in the brain of the goldfish. *In* C. J. J. Richter and H. J. Th. Goos (eds.), *Proceedings of the international symposium on reproductive physiology of fish. Wageningen, the Netherlands 2–6 August 1982*, p. 56. Centre for Agricultural Publishing and Documentation, Wageningen.

Keefer, D. A. and W. E. Stumpf. 1975*a*. Atlas of estrogen-concentrating cells in the central nervous system of the squirrel monkey. J. Comp. Neurol. 160:419–442.

Keefer, D. A. and W. E. Stumpf. 1975*b*. Estrogen localization in the primate brain. *In* W. E. Stumpf and L. D. Grant (eds.), *Anatomical neuroendocrinology*, pp. 153–165. Karger, Basel.

Kelley, D. B. 1980. Auditory and vocal nuclei in the frog brain concentrate sex hormones. Science 207:553–555.

Kelley, D. B. 1982. Female sex behaviors in the South African clawed frog, *Xenopus laevis*: Gonadotropin-releasing, gonadotropic and steroid hormones. Hormones Behav. 16:158–174.

Kelley, D. B., I. Lieberburg, B. S. McEwen, and D. W. Pfaff. 1978. Autoradiographic and biochemical studies of steroid hormone-concentrating cells in the brain of *Rana pipiens*. Brain Res. 140:287–305.

Kelley, D. B., J. I. Morrell, and D. W. Pfaff. 1975. Autoradiographic localization of hormone-concentrating cells in the brain of an amphibian, *Xenopus laevis*. I. Testosterone. J. Comp. Neurol. 164:47–62.

Kelley, D. B. and D. W. Pfaff. 1978. Generalizations from comparative studies on neuroanatomical and endocrine mechanisms of sexual behaviour. *In* J.

Hutchison (ed.), *Biological determinants of sexual behavior*, pp. 225–254. Wiley-Interscience, New York.

Kim, Y. S., W. E. Stumpf, F. A. Reid, M. Sar, and M. E. Selzer. 1980. Estrogen target cells in the forebrain of river lamprey, *Ichthyomyzon unicuspis*. J. Comp. Neurol. 191:607–613.

Kim, Y. S., W. E. Stumpf, and M. Sar. 1978. Topography of estrogen target cells in the forebrain of goldfish, *Carassius auratus*. J. Comp. Neurol. 182:611–620.

Kim, Y. S., W. E. Stumpf, and M. Sar. 1979. Topographical distribution of estrogen target cells in the forebrain of platyfish, *Xiphophorus maculatus*, studied by autoradiography. Brain Res. 170:43–59.

Kim, Y. S., W. E. Stumpf, and M. Sar. 1981a. Anatomical distribution of estrogen target neurons in turtle brain. Brain Res. 230:195–204.

Kim, Y. S., W. E. Stumpf, M. Sar, F. A. Reid, M. E. Selzer, and A. W. Epple. 1981b. Autoradiographic studies of estrogen target cells in the forebrain of larval lamprey, *Petromyzon marinus*. Brain Res. 210:53–60.

Krieger, M. S., J. I. Morell, and D. W. Pfaff. 1976. Autoradiographic localization of estradiol-concentrating cells in the female hamster brain. Neuroendocrinology 22:193–205.

Kubo, S., K. Watanabe, Y. Ibata, and Y. Sano. 1979. LH-RH neuron system of the newt by immunohistochemical study. Arch. Histol. Jap. 42:235–242.

Kyle, A. L. and R. E. Peter. 1982. Effects of forebrain lesions on spawning behaviour in the male goldfish. Physiol. Behav. 28:1103–1109.

Larsell, O. 1918. Studies on the nervus terminalis: Mammals. J. Comp. Neurol. 30:3–68.

Larsell, O. 1919. Studies on the nervus terminalis: Turtle. J. Comp. Neurol. 30:423–443.

Lehman, M. N. and S. S. Winans. 1982. Vomeronasal and olfactory pathways to the amygdala controlling male hamster sexual behavior: Autoradiographic and behavioral analyses. Brain Res. 240:27–41.

Liposits, Z., L. Nagy, and G. Sétáló. 1982. Frontal deafferentation of the mediobasal hypothalamus in the neonatal rat and its effects on the preoptico-infundibular LHRH-tract. Cell Tiss. Res. 225:179–187.

Liposits, Z., and G. Sétáló. 1980. Descending luteinizing hormone-releasing hormone (LH-RH) nerve fibers to the midbrain of the rat. Neurosci. Letters. 20:1–4.

McKibben, P. S. 1911. The nervus terminalis in urodele amphibia. J. Comp. Neurol. 21:261–309.

McNeill, T. H., G. P. Kozlowski, J. H. Abel, Jr., and E. A. Zimmerman. 1976. Neurosecretory pathways in the mallard duck (*Anas platyrhynchos*) brain: Localization by aldehyde fuchsin and immunoperoxidase techniques for neurophysin (NP) and gonadotropin releasing hormone (Gn-RH). Endocrinology 99:1323–1332.

Marshall, P. E. and P. C. Goldsmith. 1980. Neuroregulatory and neuroendocrine GnRH pathways in the hypothalamus and forebrain of the baboon. Brain Res. 193:353–372.

Martinez-Vargas, M. C., D. A. Keefer, and W. E. Stumpf. 1978. Estrogen localization in the brain of the lizard, *Anolis carolinensis*. J. Exp. Zool. 205:141–147.

Martinez-Vargas, M. C., W. E. Stumpf, and M. Sar. 1975. Estrogen localization in the dove brain. Phylogenetic considerations and implications for nomenclature. *In* W. E. Stumpf and L. D. Grant (eds.), *Anatomical neuroendocrinology*, pp. 166–175. Karger, Basel.

Martinez-Vargas, M. C., W. E. Stumpf, and M. Sar. 1976. Anatomical distribution of estrogen target cells in the avian CNS: A comparison with the mammalian CNS. J. Comp. Neurol. 167:83–104.

Merchenthaler, I., G. Kovács, G. Lovász, and G. Sétáló. 1980. The preoptico-infundibular LH-RH tract of the rat. Brain Res. 198:63–74.

Moore, F. L., L. J. Miller, S. P. Spielvogel, T. Kubiak, and K. Folkers. 1982. Luteinizing hormone-releasing hormone involvement in the reproductive behavior of a male amphibian. Neuroendocrinology 35:212–216.

Morrell, J. I., A. Ballin, and D. W. Pfaff. 1977. Autoradiographic demonstration of the pattern of ^{3}H-estradiol concentrating cells in the brain of a carnivore, the mink, *Mustela vison*. Anat. Rec. 189:609–624.

Morrell, J. I., D. Crews, A. Ballin, A. Morgentaler, and D. W. Pfaff. 1979. ^{3}H-Estradiol, ^{3}H-testosterone and ^{3}H-dihydrotestosterone localization in the brain of the lizard *Anolis carolinensis*: An autoradiographic study. J. Comp. Neurol. 188:201–224.

Morrell, J. I., D. B. Kelley, and D. W. Pfaff. 1975a. Sex steroid binding in the brains of vertebrates. *In* K. M. Knigge, D. E. Scott, H. Kobayashi, and S. Ishii (eds.), *Brain-endocrine interaction II*, pp. 230–256. Karger, Basel.

Morrell, J. I., D. B. Kelley, and D. W. Pfaff. 1975b. Autoradiographic localization of hormone-concentrating cells in the brain of an amphibian, *Xenopus laevis*. II. Estradiol. J. Comp. Neurol. 164:63–78.

Morrell, J. I. and D. W. Pfaff. 1978. A neuroendocrine approach to brain function: Localization of sex steroid concentrating cells in vertebrate brains. Amer. Zool. 18:447–460.

Morrell, J. I. and D. W. Pfaff. 1981. Autoradiographic techniques for steroid hormone localization: Application to the vertebrate brain. *In* N. T. Adler (ed.), *Neuroendocrinology of reproduction: Physiology and behavior*, pp. 519–532. Plenum Press, New York.

Morrell, J. I., T. D. Wolinsky, M. S. Krieger, and D. W. Pfaff. 1982. Autoradiographic identification of estradiol-concentrating cells in the spinal cord of the female rat. Exp. Brain Res. 45:144–150.

Moss, R. L., P. Riskind, and C. S. Dudley. 1979. Effects of LH-RH on sexual activities in animal and man. *In* R. Collu, A. Barbeau, J. R. Ducharme, and J. G. Rochefort (eds.), *Central nervous system effects of hypothalamic and other peptides*, pp. 345–366. Raven Press, New York.

Münz, H., B. Claas, W. E. Stumpf, and L. Jennes. 1982. Centrifugal innervation of the retina by luteinizing hormone releasing hormone (LHRH)-immunoreactive telencephalic neurons in teleostean fishes. Cell Tiss. Res. 222:313–323.

Münz, H., W. E. Stumpf, and L. Jennes. 1981. LHRH systems in the brain of platyfish. Brain Res. 221: 1–13.

Northcutt, R. G. 1981. Evolution of the telencephalon in nonmammals. Ann. Rev. Neurosci. 4:301–350.

Northcutt, R. G. and M. R. Braford, Jr. 1980. New observations on the organization and evolution of the telencephalon of actinopterygian fishes. *In* S. O. E. Ebbesson (ed.), *Comparative neurology of the telencephalon*, pp. 41–98. Plenum Press, New York.

Northcutt, R. G. and E. Kicliter. 1980. Organization of the amphibian telencephalon. *In* S. O. E. Ebbesson (ed.), *Comparative neurology of the telencephalon*, pp. 203–255. Plenum Press, New York.

Nozaki, M. and H. Kobayashi. 1979. Distribution of LHRH-like substance in the vertebrate brain as revealed by immunohistochemistry. Arch. Histol. Jap. 42:201–219.

Nozaki, M. and H. Kobayashi. 1980. LH-RH-like substance in the brain of lower vertebrates. *In* D. S. Farner and K. Lederis (eds.), *Neurosecretion: Molecules, cells, systems*, pp. 452–453. Plenum Press, New York.

Oksche, A. 1978. Evolution, differentiation and organization of hypothalamic systems controlling reproduction. Neurobiological concepts. *In* D. E. Scott, G. P. Kozlowski, and A. Weindl (eds.), *Brain endocrine interaction III Neural hormones and reproduction*, pp. 1–15. Karger, Basel.

Pan, C.-H., M.-Q. Feng, N.-C. Ling, S. Pao, W.-Q. Xu, G.-X. Xu, and R.-C. Shen. 1979. Immunocytochemical studies on gonadotropin releasing hormone (GnRH) secretory nucleus of the carp (*Cyprinus carpio*). Acta Biol. Exper. Sinica 12:305–310. (Original paper was not obtained—information was quoted from Peter, 1982.)

Parvizi, N. and F. Ellendorff. 1980. Gonadal steroids in the amygdala—differential effects on LH. Brain Res. 195:363–372.

Peter, R. E. 1982. Neuroendocrine control of reproduction in teleosts. Can. J. Fish. Aquat. Sci. 39: 48–55.

Peter, R. E. and V. E. Gill. 1975. A sterotaxic atlas and technique for fore-brain nuclei of the goldfish, *Carassius auratus*. J. Comp. Neurol. 159:69–102.

Pfaff, D. W. 1980. *Estrogen and brain function.* Springer-Verlag, New York.

Pfaff, D. W., J. L. Gerlach, B. S. McEwen, M. Ferin, P. Carmel, and E. A. Zimmerman. 1976. Autoradiographic localization of hormone-concentrating cells in the brain of the female rhesus monkey. J. Comp. Neurol. 170:279–294.

Pfaff, D. and M. Keiner. 1973. Atlas of estradiol-concentrating cells in the central nervous system of the female rat. J. Comp. Neurol. 151:121–158.

Phillips, H. S., B. T. Ho, and J. G. Linner. 1982. Ultrastructural localization of LH-RH-immuno-reactive synapses in the hamster accessory olfactory bulb. Brain Res. 246:193–204.

Phillips, H. S., G. Hostetter, B. Kerdelhué, and G. P. Kozlowski. 1980. Immunocytochemical localization of LHRH in central olfactory pathways of hamster. Brain Res. 193:574–579.

Sakuma, Y. and D. W. Pfaff. 1980. LH-RH in the mesencephalic central grey can potentiate lordosis reflex of female rats. Nature 283:566–567.

Sakuma, Y. and D. W. Pfaff. 1983. Modulation of the lordosis reflex of female rats by LHRH, its antiserum and analogs in the mesencephalic central gray. Neuroendocrinology 36:218–224.

Sar, M. and W. E. Stumpf. 1975a. Distribution of androgen-concentrating neurons in rat brain. *In* W. E. Stumpf and L. D. Grant (eds.), *Anatomical neuroendocrinology*, pp. 120–133. Karger, Basel.

Sar, M. and W. E. Stumpf. 1975b. Cellular localization of progestin and estrogen in guinea pig hypothalamus by autoradiography. *In* W. E. Stumpf and L. D. Grant (eds.), *Anatomical neuroendocrinology*, pp. 142–152. Karger, Basel.

Schreibman, M. P., H. Margolis-Kazan, L. Halpern-Sebold, P. A. O'Neill, and F. Caracheo. 1982. An LHRH containing center in the brain, connecting olfactory and reproductive systems. Amer. Zool. 22:856. (Abstr.)

Schreibman, M. P., L. R. Halpern, H. J. Th. Goos, and H. Margolis-Kazan. 1979. Identification of luteinizing hormone-releasing hormone (LH-RH) in the brain and pituitary gland of a fish by immunocytochemistry. J. Exp. Zool. 210:153–160.

Schreibman, M. P., L. Halpern-Sebold, M. Ferin, H. Margolis-Kazan, and H. F. Th. Goos. 1983. The effect of hypophysectomy and gonadotropin administration on the distribution and quantity of LH-RH in the brains of platyfish: A combined immunocytochemistry and radioimmunoassay study. Brain Res. 267:293–300.

Schwanzel-Fukuda, M. and A. J. Silverman. 1980. The nervus terminalis of the guinea pig: A new luteinizing hormone-releasing hormone (LHRH) neuronal system. J. Comp. Neurol. 191:213–225.

Sheridan, P. J. 1978. Localization of androgen- and estrogen-concentrating neurons in the diencephalon and telencephalon of the mouse. Endocrinology 103:1328–1334.

Sheridan, P. J., N. Hagino, and F. J. Weaker. 1982. Androgen-concentrating cells in the periventricular brain of the female rhesus monkey. J. Comp. Neurol. 207:93–98.

Sheridan, P. J. and F. J. Weaker. 1982. Androgen receptor systems in the brain stem of the primate. Brain Res. 235:225–232.

Shivers, B. D., R. E. Harlan, J. I. Morrell, and D. W. Pfaff. 1983. Immunocytochemical localization of luteinizing hormone-releasing hormone in male and female rat brains. Neuroendocrinology 36: 1–12.

Silverman, A. J., J. L. Antunes, G. M. Abrams, G. Nilaver, R. Thau, J. A. Robinson, M. Ferin, and L. C. Krey. 1982. The luteinizing hormone-releasing hormone pathways in rhesus (*Macaca mulatta*) and pigtailed (*Macaca nemestrina*) mon-

keys: New observations on thick, unembedded sections. J. Comp. Neurol. 211:309–317.

Silverman, A. J. and L. C. Krey. 1978. The luteinizing hormone-releasing hormone (LH-RH) neuronal networks of the guinea pig brain. I. Intra- and extra-hypothalamic projections. Brain Res. 157:233–246.

Silverman, A. J., L. C. Krey, and E. A. Zimmerman. 1979. A comparative study of the luteinizing hormone releasing hormone (LHRH) neuronal networks in mammals. Biol. of Reprod. 20:98–110.

Silverman, A. J. and E. A. Zimmerman. 1978. Pathways containing luteinizing hormone-releasing hormone (LHRH) in the mammalian brain. *In* D. E. Scott, G. P. Kozlowski, and A. Weindl (eds.), *Brain-endocrine interaction III. Neural hormones and reproduction*, pp. 83–96. Karger, Basel.

Sower, S. A., W. W. Dickhoff, A. Gorbman, W. W. Vale, and J. E. Rivier. 1982. Actions of analogues of luteinizing hormone-releasing hormone (LH-RH) in the sea lamprey. Amer. Zool. 22:855. (Abstract.)

Springer, A. D. 1983. Centrifugal innervation of goldfish retina from ganglion cells of the nervus terminalis. J. Comp. Neurol. 214:404–415.

Stacey, N. E. and A. L. Kyle. 1983. Effects of olfactory tract lesions on sexual and feeding behavior in the goldfish. Physiol. and Behav. 30:621–628.

Stell, W. K., S. E. Walker, K. S. Chohan, and A. K. Ball. 1984. The goldfish nervus terminalis: An LHRH- and FMRFamide-immunoreactive olfactoretinal pathway. Proc. Natl. Acad. Sci. U.S.A. 81:940–944.

Sterling, R. J. and P. J. Sharp. 1982. The localisation of LH-RH neurones in the diencephalon of the domestic hen. Cell Tiss. Res. 222:283–298.

Stumpf, W. E. 1970a. Tissue preparation for the autoradiographic localization of hormones. *In* G. L. Wied and G. F. Bahr (eds.), *Introduction to quantitative cytochemistry, Vol. 2*, pp. 507–526. Academic Press, New York.

Stumpf, W. E. 1970b. Estrogen-neurons and estrogen-neuron systems in the periventricular brain. Am. J. Anat. 129:207–218.

Stumpf, W. E. and M. Sar. 1978. Anatomical distribution of estrogen, androgen, progestin, corticosteroid and thyroid hormone target sites in the brain of mammals: Phylogeny and ontogeny. Amer. Zool. 18:435–445.

Stumpf, W. E., M. Sar, and D. A. Keefer. 1975. Atlas of estrogen target cells in rat brain. *In* W. E. Stumpf and L. D. Grant (eds.), *Anatomical neuroendocrinology*, pp. 104–119. Karger, Basel.

Van Wijhe, J. W. 1919. On the nervus terminalis from man to amphioxus. Kon. Akad. Weten. Amsterdam, Sect. A. 21:172–182.

Warembourg, M. 1977a. Fixation des steroides au niveau du système nerveux central et de l'hypophyse chez différents mammifères. Ann. d'Endocrinol. 38:41–54.

Warembourg, M. 1977b. Radioautographic localization of estrogen-concentrating cells in the brain and pituitary of the guinea pig. Brain Res. 123:357–362.

Warembourg, M. 1977c. Topographical distribution of estrogen-concentrating cells in the brain and pituitary of the squirrel monkey. Neurosci. Letters 5:315–319.

Witkin, J. W., C. M. Paden, and A. J. Silverman. 1982. The luteinizing hormone-releasing hormone (LHRH) systems in the rat brain. Neuroendocrinology 35:429–438.

Wood-Gush, D. G. M., G. A. S. Langley, A. F. Leitch, M. J. Gentle, and A. B. Gilbert. 1977. An autoradiographic study of sex steroids in the chicken telencephalon. Gen. Comp. Endocrinol. 31:161–168.

Zigmond, R. E., R. A. Detrick, and D. W. Pfaff. 1980. An autoradiographic study of the localization of androgen concentrating cells in the chaffinch. Brain Res. 182:369–381.