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# A Comparative Study of the Luteinizing Hormone Releasing Hormone (LHRH) Neuronal Networks in Mammals[1]

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## ABSTRACT

The development of immunocytochemical procedures utilizing antisera to synthetic luteinizing hormone releasing hormone (LHRH) has permitted the identification and description of neurons which synthesize, store and secrete this neurohormone. This paper will present an overview of published studies for several mammalian species in an attempt to characterize and describe interspecies similarities and differences in LHRH distribution. In studies employing a combination of radiofrequency lesions and immunocytochemical assessments of lesion effects, at least 6 separate LHRH neuronal networks have been identified and traced in the guinea pig brain. In view of this work, this species will be used as the representative mammal against which distribution patterns of immunoreactive LHRH-containing neurons in mouse, hamster and primates will be compared. Finally, the physiological roles of these neuroendocrine networks in the regulation of anterior pituitary function and control of sexual behavior will be discussed.

## INTRODUCTION

The distribution of luteinizing hormone releasing hormone (LHRH) perikarya and the pathways of their axons within the central nervous system have been investigated by LHRH bioassay and radioimmunoassay of identified brain regions as well as by immunocytochemistry. This latter approach has been of primary value to both the neuroendocrinologist and neuroanatomist for two reasons. At the light microscopic level, immunocytochemistry has provided the means for determining precisely the localization and morphology of those neurons involved in biosynthesis of LHRH including their axonal projections and sites of termination. Electron microscopic immunocytochemistry has provided data on the mechanism of intracellular packaging from synthesis to release. Many light microscopic immunocytochemical studies on the distribution of LHRH have been reported for a variety of mammalian species. Unfortunately, these studies have employed an array of different antisera as well as a variety of experimental techniques (immunofluorescence vs unlabeled antibody enzyme method). As a result, one cannot assume that any one study provides a complete map of LHRH neurons. Furthermore, this plethora of experimental procedures makes it difficult to assess potential interspecies variations in LHRH neuron distribution.

In this paper we shall attempt to summarize the literature on the morphology and distribution of LHRH neurons in the brains of several mammalian species.

To this end, we shall use the guinea pig as a representative mammal. We have studied this species in detail, having described not only the origin of LHRH-containing neuronal networks within the brain but also the neuroendocrine role of these networks in the regulation of gonadotropin secretion from the pituitary gland (Silverman and Krey, 1978; Krey and Silverman, 1978).

## METHODS AND MATERIALS

For the data reported from this laboratory the following immunocytochemical procedures were used. Brains were rapidly removed from guinea pigs (Topeka or Hartley strain), mice (Swiss albino or New Zealand white) and golden hamsters after decapitation, dissected

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and fixed in Bouin's solution for 48 h at 4°C. Rhesus monkeys were perfused through the heart with Bouin's solution following a saline flush during ketamine or sodium pentabarbitol anesthesia. One monkey brain was fixed by immersion in Bouin's solution and another by perfusion with 10% buffered formalin. Blocks of tissue were dissected and immersed for 3–21 days at 4°C in the perfusion fixative. Following fixation, all tissue was dehydrated in graded alcohols, cleared in xylene and embedded in paraffin. Some brains were cleared in cedarwood oil prior to xylene; this treatment did not affect later staining procedures. Serial 6 μm sections were mounted individually on glass slides in a gelatin water bath and dried overnight at 40°C. Representative sections were stained with cresyl violet for orientation.

Immunocytochemical localization was carried out by sequential application of 0.75% gelatin (10 min); primary antiserum to LHRH (48 h, 4°C); sheep antirabbit globulin serum (1:100, 30 min); the peroxidase-antiperoxidase complex (1:400, 60 min); 3,3′ diaminobenzidine with hydrogen peroxide (5–15 min, 20°C). Five different antisera to LHRH were used: 1) rabbit E (bled 5/74); 2) rabbit F (bled 11/7/73); 3) rabbit 4305B (bled 1/8/76); 4) rabbit 2189-7 and 5) rabbit 38. Antisera 1 and 2 were provided by S. Sorrentino, Jr., 3 by L. A. Sternberger, 4 by B. Kerdelhue and 5 by G. Niswender. Antibodies to bovine serum albumin were removed from sera 1, 2 and 5 by precipitation and centrifugation or by affinity chromatography.

Both immunological and methodological controls have been described in detail in previous publications (Silverman, 1976; Silverman et al., 1977; Zimmerman et al., 1974). All reaction products were eliminated by the addition of small quantities of synthetic LHRH (Abbott) to each of the primary antisera for 24 h prior to its application to the tissue sections.

For studies from other laboratories, the reader is referred to the original papers for descriptions of immunocytochemical techniques, antisera and controls.

Electron microscopic immunocytochemistry performed in this laboratory uses essentially the same staining procedure as for light microscopy (Silverman and Desnoyers, 1976). Tissue is fixed either by freeze-substitution or with a mixture of glutaraldehyde and paraformaldehyde (Silverman and Desnoyers, 1976). The tissue is embedded in Araldite 6005 and the reactions are carried out on ultra-thin sections etched with hydrogen peroxide. The reaction product is made electron dense by exposure of the section to 4% osmium tetroxide.

### *Neurosurgical Procedures*

In the guinea pig studies lesions were made with a Cramer LM-3 radiofrequency lesion maker (Silverman and Krey, 1978; Krey and Silverman, 1978). Electrode placements were performed under pentobarbital anesthesia according to the atlas of Poulain (1974). In sham lesioned animals the stainless steel electrode was simply lowered into the hypothalamus. Lesions were placed bilaterally in the arcuate and suprachiasmatic nuclei, the mammillary body complex, the medial preoptic area, the medial septal nucleus and medial amygdaloid nucleus. Additional animals were subjected to fornix transections performed by suction. Animals were decapitated 3–5 weeks following lesion placement. The extent of the lesions was later verified by examination of cresyl violet stained, sagittal sections through the medial-lateral extent of the brain.

Blood samples for radioimmunoassay of gonadal steroids and gonadotropins were collected prior to and following lesion placement (Krey and Silverman, 1978).

## RESULTS

### *Morphology*

The localization of LHRH neurons in a tissue section by immunocytochemical techniques depends on a sufficient concentration of the peptide to react with the antibody under experimental conditions. The level of immunoreactive LHRH remaining in a section depends not only on the endocrine state of the animal, but also on the fixation, dehydration and embedding techniques employed. Techniques can influence not only the extraction of LHRH from tissue but also the antigenicity of the peptide that remains *in situ* (Goldsmith and Ganong, 1975; Silverman and Desnoyers, 1976). Finally the antisera themselves have variable sensitivities which can effect the observed pattern of immunoreactive cells (Silverman and Zimmerman, 1978; Hoffman et al., 1978). Despite the aforementioned difficulties, LHRH immunoreactive perikarya have been observed in guinea pig (Barry et al., 1974; Barry and Dubois, 1974; Silverman, 1976), cat and dog (Barry and Dubois, 1975), mouse (Zimmerman et al., 1974), rabbit (Barry, 1976a), several species of primates (Barry et al., 1975; Barry and Carette, 1975; Silverman et al., 1977) including human fetus (Bugnon et al., 1977) and man (Barry, 1976b).

The morphology of LHRH-containing cell bodies appears comparable in all species studied but is more or less defined by the amount of immunoreactive material especially in the dendrites. The size varies from 10–40 μm in diameter depending on the species (Barry, 1979). Immunoreactive dendrites are few in number (averaging 1–4) and poorly branched. Representative examples from various species are shown in Figures 1A-1D. In some cases axons with beaded varicosities are also visible extending from the cell body (Fig. 1A).

At the electron microscope level LHRH neurons have been examined in two species. In the study of Mazzuca (1977), cells were described in the organum vasculosum of the lamina terminalis (OVLT) with positive granules of 90–130 nm diameter. These cells had a well-

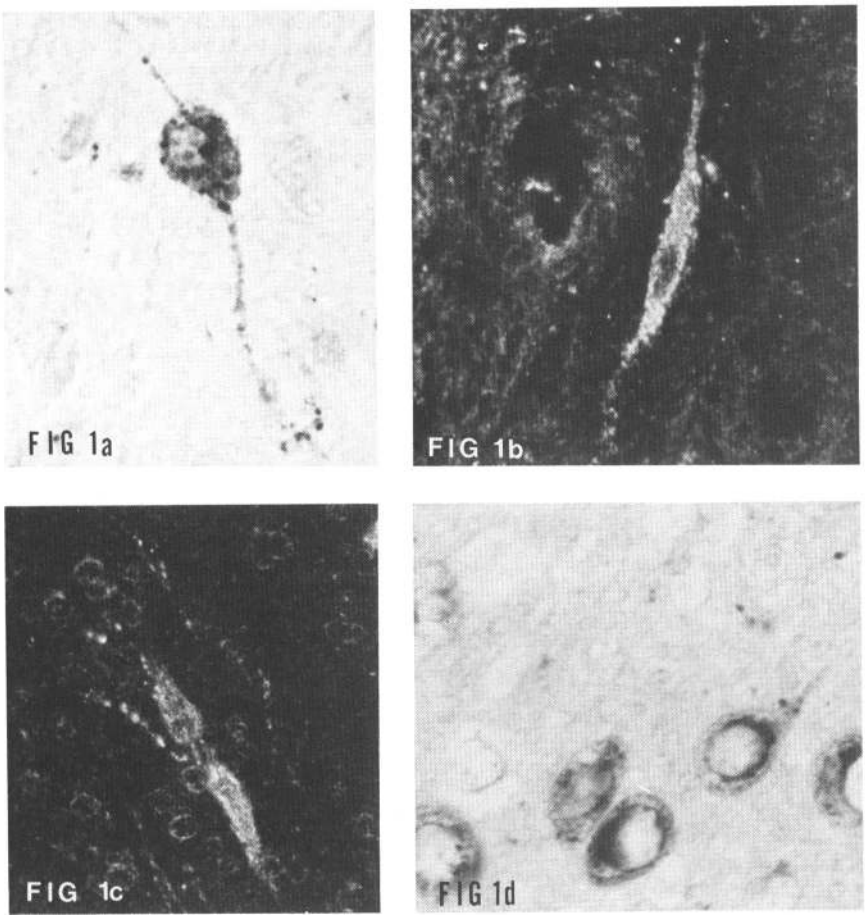


FIG. 1. LHRH positive perikarya from different mammals. A) Bright field micrograph of neuron in the medial preoptic area of the guinea pig. Note the beaded axon extending from the cell body (arrow). Section was reacted with antiserum 2. × 400. B) Dark field micrograph of neuron in the lamina terminalis of the rhesus monkey. Section was reacted with antiserum 1. × 400. C) Dark field micrograph of neurons in the medial septal nucleus of the mouse. Section was reacted with antiserum 4. × 400. D) Bright field micrograph of neurons in the arcuate nucleus of the hamster. Note the intense perinuclear staining. Section was reacted with antiserum 2. (Micrograph provided by G. E. Pickard.) × 700.

developed rough endoplasmic reticulum associated with a large Golgi area. In the work of Bugnon et al. (1977), LHRH neurons in the human fetus were identified by light microscopic examination of immunologically stained semithin sections. Their ultrastructure was then studied in serial ultrathin section and were characterized by large numbers of neurosecretory granules.

In the guinea pig median eminence LHRH axons within the palisade zone contain immunoreactive granules of 9–120 nm (Fig. 2); peptide-containing granules in terminals abutting on the perivascular space of the portal plexus are 40–80 nm in diameter (Silverman and Desnoyers, 1976). The localization of LHRH in granules of various sizes in the axons and terminals of the median eminence have been reported for rat (Pelletier et al., 1975; Goldsmith and Ganong, 1975) and duck (Calas et al., 1974).

*Distribution of LHRH*

We shall discuss in detail the distribution of this peptide in the guinea pig, primate, mouse and hamster as determined by immunocytochemistry. Other mammalian and nonmammalian species have been reviewed by Barry (1979) and Hoffman et al., 1978).

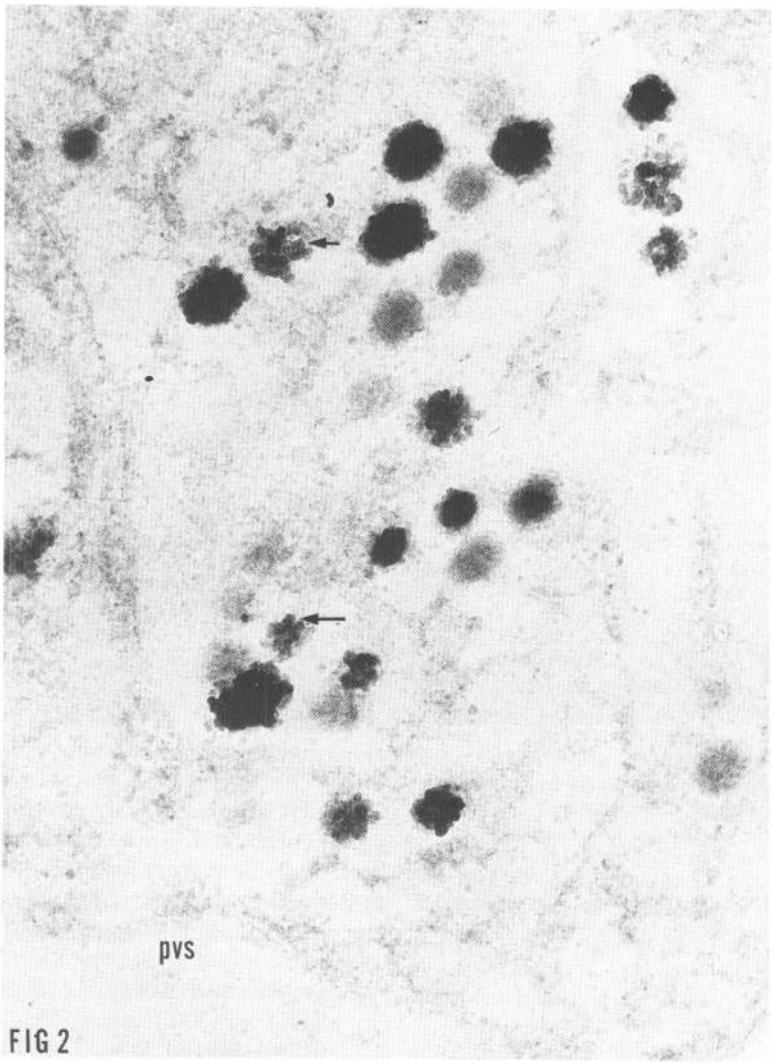


FIG. 2. Electron micrograph of LHRH positive axon terminal abutting on the perivascular space (pvs) of a portal capillary in the median eminence of the guinea pig. Arrows indicate molecules of the peroxidase-antiperoxidase complex. X 94,000.

*Guinea Pig*

LHRH reactive perikarya were first described in the guinea pig by Barry et al. (1974). The distribution of these neurons is shown diagrammatically in Fig. 3. We have described four distinct collections of LHRH containing perikarya in this species (Silverman and Krey, 1978). One was present in the medial basal hypothalamus, predominately in the arcuate nucleus. A very small number of cells in this area was observed in the adult (Silverman, 1976), but large numbers were observed in the developing fetus (Schwanzel-Fukada and Silverman, 1978). A second group of LHRH neurons was located in the medial preoptic area with the majority of cells lying in the medial preoptic nucleus; reactive cells associated with this group were also present in the periventricular preoptic nucleus, bed nucleus of the stria terminalis, preoptic portion of the suprachiasmatic nucleus and lamina terminalis. In the fetus, a few cells were also found in the lateral preoptic nucleus. A third group of LHRH containing neurons was present scattered in the septal area with the majority of cells lying in the medial septal nucleus; smaller numbers were found in the

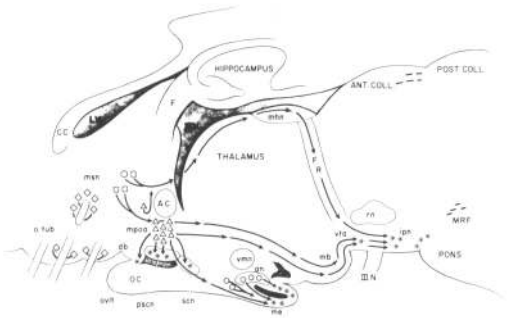


FIG. 3. Camera lucida drawing of a parasagittal section of a guinea pig brain showing the location of LHRH neurons and their projections. Terminal fields are indicated with an asterisk (*). Positive neurons in the medial basal hypothalamus including the arcuate nucleus (an) project to the zona externa of the median eminence (me). Others in the medial preoptic area (mpoa) project to the organum vasculosum of the lamina terminalis (ovlt), preoptic portion of the suprachiasmatic nucleus (pscn) and retrochiasmatic portion of the suprachiasmatic nucleus (scn). LHRH neurons in this region also project to a small extent to the zona externa on the lateral and ventral surfaces of the infundibular stalk and to the zona interna throughout the extent of the median eminence and stalk. MPOA neurons also project through the dorsomedial hypothalamus to converge at the level of the mammillary body (mb) and especially the fiber capsule under the mb. These fibers continue down the mammillary peduncle to the ventral tegmental area (vta). Some LHRH neurons in the medial septal nucleus (msn) follow the same route to the vta as those in the mpoa. Others project via the stria medularis, through the medial habenular nucleus (mhn) down the fasciculus retroflexus (FR) to the medial and caudal portions of the interpeduncular nucleus (ipn). Other LHRH neurons ( ) are seen clustered around large blood vessels in the medial septal nucleus, nucleus of the diagonal band (db) and olfactory tubercle (o. tub.); their axons appear to terminate primarily around these blood vessels. Scattered axons of unknown origin are found in the anterior colliculus (Ant. Coll.) and midbrain reticular formation (MRF). Additional abbreviations: CC = corpus callosum; Post. Coll. = posterior colliculus; rn = red nucleus; vm = ventromedial nucleus.

lateral septal nucleus and nucleus and tract of the diagonal band of Broca. The fourth group was congregated in small clusters in the anterior perforated substance; these cells were found in close association with the walls of the large blood vessels in this region. Barry (1976c) has also reported LHRH-containing neurons in the amygdala and mesencephalon of the guinea pig which we have not yet seen.

The fiber tracts originating from these cell bodies have been extensively described by this laboratory (Silverman and Krey, 1978; Krey and Silverman, 1978) and by Barry and his collaborators (Barry et al., 1974). They are shown diagrammatically in Fig. 3. The most heavily labeled fiber tracts, i.e., those containing the most fibers, were present in the diencephalon. They were located bilaterally in: A) the medial preoptic area, extending between the anterior commissure and the organum vasculosum of the lamina terminalis (OVLT), the preoptic portion of the suprachiasmatic nucleus and to a much lesser extent in the retrochiasmatic portion of the suprachiasmatic nucleus and B) in the medial basal hypothalamus terminating in the median eminence and infundibular stalk. A third group of axons (Tract C) crossed the hypothalamus in a very diffuse manner. Beginning in the medial preoptic area, they appeared to pass through the dorsomedial hypothalamus, turn ventrally and caudally through the medial mammillary nucleus and then enter the fiber capusle under the mammillary complex. The fibers continued via the mammillary peduncle into the ventral tegmental area where they appeared to terminate on neurons. These fibers were scattered from midline to 0.5 mm from the midline.

LHRH positive fibers were also observed in extrahypothalamic areas. Scattered fibers were present in the septum just dorsal and rostral to the anterior commissure. Many were situated in the precommisural component of the stria terminalis; others were present in the triangular and medial septal nuclei and diagonal band of Broca.

Fibers were also present in and under the stria medularis thalami, in the medial habenular nucleus, fasciculus retroflexus (fiber tract connecting the habenula with the interpeduncular nucleus of the midbrain) and superior colliculus. Those in the fasciculus retroflexus appeared to extend into the interpeduncular nucleus where they terminated in either the midportion of the interpeduncular nucleus around branches of the posterior communicating artery or near neurons located at the interpeduncular-pontine border.

Several small clusters of intensely labeled fibers were observed in conjunction with the positive cells in the anterior perforated substance (Fig. 4). These fibers passed along side the large blood vessels of this region. Very few fibers were observed to extend from one group toward another.

Using higher concentration of primary

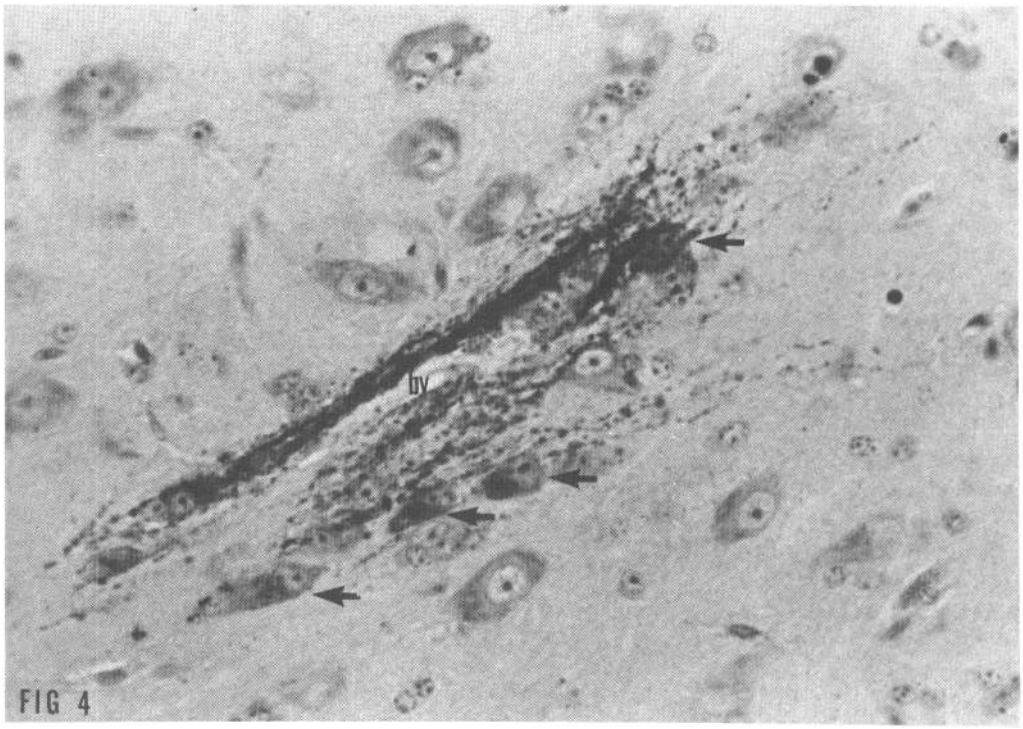


FIG. 4. LHRH positive neurons and axons in the anterior perforated substance of the guinea pig. Positive perikarya (arrows) and their axons tend to lie very near to large blood vessels (bv) in this region. The section was reacted with antiserum 3. × 400.

antiserum, occassional scattered fibers were also observed in: 1) the choroid plexus of ventricles III and IV; 2) midbrain central grey; 3) brainstem reticular formation and 4) medial amygdaloid nucleus. The latter fiber tract has been described in more detail by Leonardelli and Poulain (1977).

To determine the contribution that each of the LHRH-containing cell groups makes to the various fiber tracts as well as their possible role in the regulation of tonic gonadotropin secretion, radiofrequency lesions were placed in a variety of brain areas (Silverman and Krey, 1978; Krey and Silverman, 1978). The results of these studies are summarized below.

*Effects of lesions on LHRH distribution in the median eminence.* The effects of the lesions on the distribution of LHRH in the median eminence was studied in the male guinea pigs. In animals with fornix transections or lesions in the septal area, mammillary bodies or medial amygdaloid nucleus, LHRH distribution in the median eminence was similar to that observed in the sham lesioned controls. Furthermore, when comparisons were made between sections from animals with these lesions and the controls, the amount of immunoreactive material appeared equivalent at several dilutions of the antiserum.

In contrast, the median eminence of those animals with lesions in the arcuate nucleus contained only a few immunoreactive fibers. This was true even at a 1:2000 dilution of the antiserum.

Medial preoptic area or suprachiasmatic nucleus lesions had three distinct effects on the distribution of LHRH positive axons in the median eminence. First, these lesions reduced the number of labeled processes in the zona externa of the ventral and lateral portions of the infundibular stalk. This deficit was observed at primary antiserum dilutions of 1:2000 to 1:6000 and, therefore, probably reflects a loss of fibers. Secondly, a similarly significant loss of immunoreactive axons was apparent in the internal and subependymal zones of the median eminence throughout its rostral-caudal extent. The third effect was to reduce the amount of immunoreactive material in the axons and terminals of the external zone

of the medial dorsal portion of the stalk. When sections were stained using a 1:6000 dilution of the primary antiserum, only the terminal portion of the axons could be visualized in the lesioned animals while this same concentration labeled both the preterminal and terminal portions of the axons in the sham lesioned controls. However, with dilutions of 1:2000 to 1:1400, reaction product was visible in the preterminal axons in the lesioned animals and the medial dorsal portion of the stalk appeared similar to sham operated controls.

*Organum vasculosum of the lamina terminalis (OVLT).* Lesions which destroyed the LHRH cells in the medial preoptic area (and did not extend into the lamina terminalis or the OVLT itself) resulted in an almost complete depletion of labeled axons in the OVLT. The LHRH projection to this circumventricular organ was also substantially reduced when the lesions impinged on the lamina terminalis. All other lesions, including those in the arcuate nucleus, had no effect on the distribution of LHRH fibers in the OVLT.

*Suprachiasmatic nucleus.* A substantial number of LHRH fibers terminate in the preoptic portion of the suprachiasmatic nucleus which is just caudal to the lamina terminalis at midline. Far fewer fibers terminate in the retrochiasmatic portion. Following medial preoptic area lesions, labeled fibers were absent from the preoptic and retrochiasmatic portions of this nucleus. Other lesions had no effect.

*Mammillary body and ventral tegmental area.* Lesions in the medial preoptic area but not in the suprachiasmatic nucleus resulted in substantial decreases in the number of LHRH fibers in the ventral capsule under the mammillary complex. In addition, the LHRH terminal field in the ventral tegmental area was eliminated or reduced. The extent to which labeled fibers remained in either the mammillary body or the ventral tegmental area was directly related to the extent of damage to the medial preoptic area. Lesions within the mammillary body itself disrupted this pathway but only when the ventral fiber capsule was destroyed. Such mammillary body lesions, however, had no effect on preoptic projections to the OVLT and suprachiasmatic nucleus. Arcuate lesions resulted in varying degrees of loss of labeled fibers in the mammillary body and the tegmental area; this was dependent on the extent of damage to the ventromedial and premammillary nuclei.

*Thalamus–habenula–fasciculus retroflexus–interpeduncular nucleus.* Large lesions of the septal and preoptic area resulted in the absence of or significant decrease in labeled fibers in the anterior thalamic nucleus, stria medularis, habenula, fasciculus retroflexus and the terminal fields in the caudal aspect of the interpeduncular nucleus and the region just dorsal to the interpeduncular nucleus. Medial preoptic area lesions alone reduced the number of labeled fibers in these structures to a smaller extent.

*Anterior perforated substance.* The lesions had no effect on LHRH positive axons and cell bodies present in this area. Even large lesions in the medial septal nucleus did not disrupt the clusters of cells and fibers in the nucleus of the tract of the diagonal band and olfactory tubercle.

Because of the paucity of labeled fibers even in the sham operated animals, the effects of the various lesions on the LHRH projections to the choroid plexus, medial amygdaloid nucleus and brain stem reticular formation were not determined.

*Effects of lesions on gonadotropin secretion.* Males: The effects of the lesions on serum LH and testosterone are shown in Table 1. Animals with arcuate nucleus lesions had significantly lower serum testosterone and gonadotropin levels in addition to atrophied testes and seminal vesicles. These findings suggest a deficiency in gonadotropin secretion and this was confirmed by measurement of low circulating LH levels. In all other lesioned groups, these parameters of pituitary-gonadal function did not differ from sham operated controls. None of the lesions had any effect on adrenal weight.

Females: Female guinea pigs with lesions in the medial preoptic area, suprachiasmatic nucleus or mammillary body complex showed a postovariectomy rise in serum LH that was indistinguishable from that in the sham lesioned controls. This rise was approximately 5-fold over preovariectomy levels. Animals bearing arcuate nucleus lesions, however, had markedly attenuated gonadotropin responses. At no time did serum LH values rise above preovariectomy values. The negative feedback action of estradiol on gonadotropin release was observed in all but the animals with arcuate nucleus lesions (Krey and Silverman, 1978).

### *Primates*

In the rhesus monkey, the localization of immunoreactive cells within the hypothalamus

TABLE 1. Effects of hypothalamic and extraphyothalamic radiofrequency lesions on the pituitary-testicular axis in male guinea pigs.

| Lesion sites | Testis (mg/100 g b. w.) | | LH[a] | | Testosterone | |
|---|---|---|---|---|---|---|
| Sham (6) | 307 | 31[b] | 0.74 | 0.27 | 4.17 | 0.82 |
| Arcuate nucleus (6) | 75 | 18[c] | <0.17 | | 0.33 | 0.07[c] |
| Septum (3) | 333 | 31 | 0.79 | 0.49 | 3.54 | 0.98 |
| Preoptic area (11) | 365 | 16 | 0.71 | 0.25 | 5.84 | 0.58 |
| Suprachiasmatic nucleus (5) | 343 | 19 | 0.34 | 0.05 | 5.10 | 0.43 |
| Mammillary body (4) | 336 | 50 | 0.48 | 0.16 | 3.16 | 0.53 |
| Fornix transection (3) | 354 | 2 | 0.84 | 0.39 | 3.36 | 0.51 |
| Medial amygdala (4) | 388 | 48 | 0.35 | 0.04 | 2.39 | 0.39 |

[a] ng NIH-LH-S19/ml.

[b] mean ± SEM of number of animals in parentheses.

[c] $P \leq 0.001$ vs sham.

is similar to that in the guinea pig though somewhat more diffuse (Fig. 5) (Silverman et al., 1977). Using antisera 1, 2 or 3 we could demonstrate LHRH neurons in the pre- and pericommissural region (bed nucleus of the stria terminalis, medial preoptic nucleus), lamina terminalis and infundibular area (infundibular and premammillary nuclei, retrochiasmatic area).

Positive fibers were widely scattered and less well-organized into discrete fiber tracts than in the guinea pig. Many coursed from the commissural region ventrally to the OVLT. Others from this region extended into the septal area. Those in the infundibular region appeared to project primarily to the zona externa of the median eminence. Unlike other species, many LHRH axons continued down the infundibular stalk and terminated in the posterior pituitary gland (Fig. 6). Occasionally positive fibers were found in the medial habenular nucleus and fasciculus retroflexus. A large plexus of axons was observed in the medial mammillary nucleus near the mammillo-thalamic tract. No fibers have yet been traced into the midbrain.

Similar findings to those in the rhesus monkey have been obtained by immunofluorescence in *Macacus* (Barry et al., 1975), *Cercopithecus* (Barry et al., 1975) and *Cebus apella* (Barry and Carette, 1975). In squirrel monkey (*Saimiri sciurus,* Barry and Carette, 1975), LHRH neurons were present in the tuberoinfundibular and premammillary regions, scattered as a septo-preoptic group and in both pre- and pericommissural areas. In man (Barry, 1976b) and human fetus (Bugnon et al., 1977), positive cells are found in the medio-basal

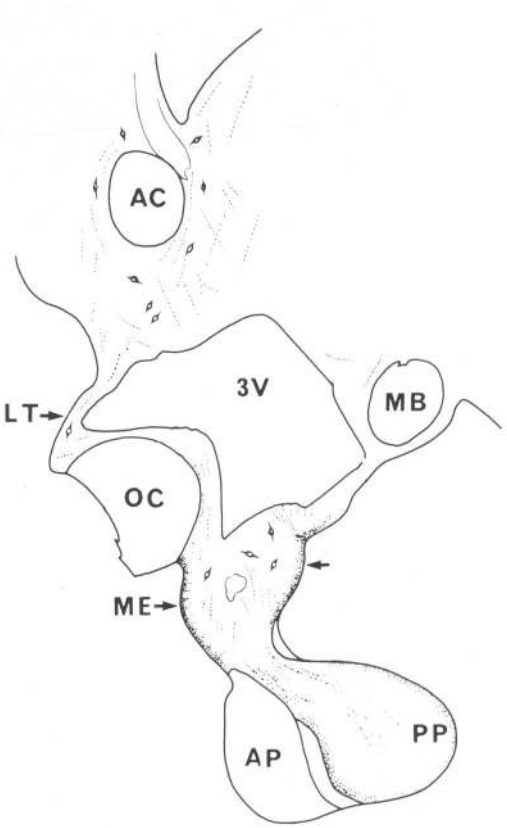


FIG. 5. Camera lucida drawing of sagittal section of the hypothalamus of a female rhesus monkey. All LHRH positive elements observed in the section are indicated. There is a heavy concentration of positive perikarya around the anterior commissure (AC) and the infundibular area. Axons can be seen extending from the pericommissural area to the lamina terminalis (LT) and from the infundibular area to the median eminence (ME) and posterior pituitary (PP). OC, optic chiasm; AP, anterior pituitary; MB, mammillary body; 3V, third ventricle.

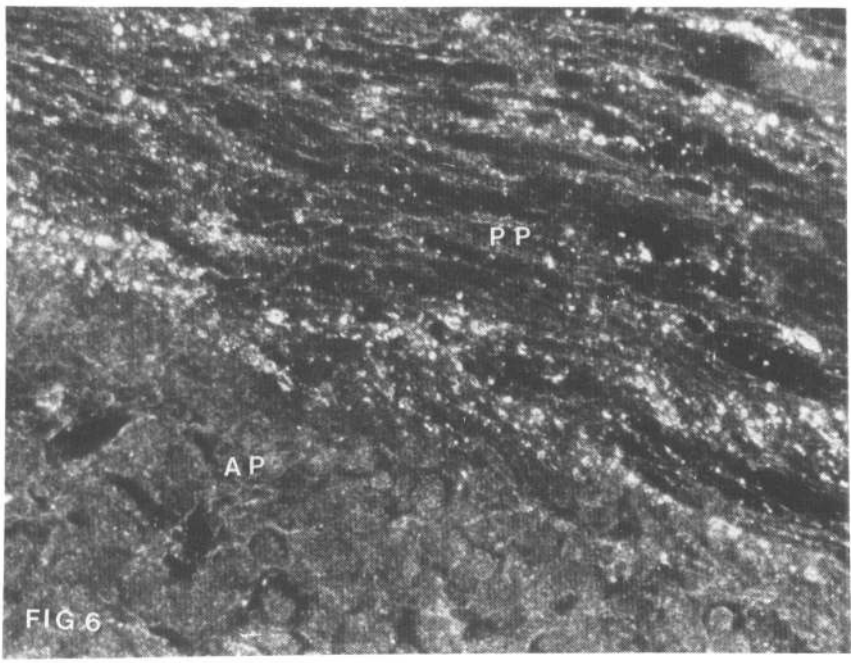


FIG. 6. Dark field micrograph of a sagittal section through the pituitary gland of a rhesus monkey. LHRH axons are present in the posterior pituitary (PP) and are particularly concentrated along the border with the anterior pituitary (AP) which is free of reaction products. × 320.

tuber (retrochiasmatic and infundibular area, post-infundibular median eminence, premamillary nucleus), in the lamina terminalis, septum and pericommissural area. A few scattered LHRH neurons were also observed in the ventromedial and dorsomedial hypothalamic nuclei.

*Mouse*

In our original studies on the distribution of LHRH in the mouse brain, we observed positive neurons in the arcuate nucleus with fibers extending into the median eminence (Zimmerman et al., 1974). LHRH terminals were also observed in the OVLT. These results could be obtained with either antiserum 5 (Zimmerman et al., 1974) or antiserum 2 (Silverman, 1976). By extending the period of incubation with antiserum 2 to 48 h, additional fibers could be observed in all hypothalamic and preoptic areas described for the guinea pig with the exception of the suprachiasmatic nucleus. Use of antisera 3 or 4 revealed reactive cell bodies in the medial preoptic area and septal area but these antisera did not react with those in the arcuate region (Silverman and Zimmerman, 1978). This is a primary example of the differential specificity of different antisera.

In the mouse, axons containing LHRH were noted in the subfornical organ and in the glomerulosa layer of the olfactory bulb. LHRH axons have not to date been observed in these structures in the guinea pig.

*Hamster*

In the golden hamster, LHRH positive neurons are observed only in the medial basal hypothalamus including the arcuate, ventromedial and premammillary nuclei (Pickard and Silverman, 1976). Even when antiserum 4, which reacts very well with medial preoptic LHRH neurons on the mouse (Silverman and Zimmerman, 1977) and rat (Hoffman et al., 1977), was tested no positive perikarya were found in the more rostral regions of the brain. The number of positive LHRH perikarya in the medial hypothalamus is much larger than that noted in other animals; however, the number of axons in the median eminence and in other hypothalamic and extrahypothalamic sites is very small. Careful examination of stained sections has revealed fiber tracts and sites of termination very similar to that observed in the guinea pig (Pickard and Silverman, 1976).

## DISCUSSION

The results from our study using radiofrequency lesions in the guinea pig have led us to postulate the existence in this species of the LHRH networks diagrammed in Fig. 3. LHRH neurons in the medial preoptic area have at least five separate projection pathways. The heaviest is that to the OVLT and suprachiasmatic nucleus, especially the preoptic portion. This OVLT projection also appears to be present in the rat (Weiner et al., 1975), rhesus monkey (Silverman et al., 1977), mouse (Zimmerman et al., 1974) and various other mammals (Barry, 1979). The termination in the suprachiasmatic nucleus does not appear to be prominent or present in species other than the guinea pig. The second pathway originating in the medial preoptic area in the guinea pig courses dorsally around the anterior commissure into the bed nucleus of the stria terminalis and surrounding septal area. Similar fiber projections can also be traced in the rhesus monkey and mouse (Silverman and Zimmerman, 1978). The third pathway is a very diffuse rostral-caudal projection through the medial mammillary nucleus and the fiber capsule under the mammillary bodies to the ventral tegmental area via the mammillary peduncle. In the guinea pig, the medial preoptic neurons also appear to contribute to the habenular-fasciculus retroflexus-interpeduncular nucleus tract (projection 4). In the fifth pathway, the preoptic LHRH neuron projection to the internal zone of the median eminence and to the external zone on the ventral and lateral surfaces of the infundibular stalk. The contribution of MPOA-LHRH neurons to the terminals in the median eminence in other species remains to be determined.

Those LHRH neurons which are found "randomly" scattered in the medial septal nucleus and nucleus and tract of the diagonal band appear to send axons to the habenula, down the fasciculus retroflexus to terminate in the caudal portion of the interpeduncular nucleus. Herkenham and Nauta (1977) have shown that the medial habenular nucleus does receive fibers from the septum and diagonal band but not from the hypothalamus or medial preoptic area. Meiback and Siegal (1977) have demonstrated that the diagonal band projects via the medial forebrain bundle to the fiber capsule under the mammillary bodies and from there to a region just dorsal to the interpeduncular nucleus. In none of these studies, however, have fibers of septal origin been observed to leave the habenula and enter the fasciculus retroflexus, terminating in the caudal and medial portion of the interpeduncular nucleus. Since the LHRH fibers which do so are extremely few in number it is possible that they might be missed by less sensitive methods.

The LHRH neuron clusters within the anterior perforated substance of the guinea pig appear to form self-contained systems. The groups of cells and fibers were observed following all lesions even those which destroyed similar groups in the septal area. These groups of cells were intimately associated with large blood vessels in this region and the LHRH axons appear to terminate in the wall of the blood vessels. These cells have not been reported in other species. In the guinea pig (Krey and Silverman, 1978) as well as in the rhesus monkey (Silverman et al., 1977) and mouse (Zimmerman et al., 1974; Silverman, 1976), the LHRH cell bodies in the medial basal hypothalamus (arcuate nucleus and retrochiasmatic area) appear to project primarily to the median eminence. Arcuate nucleus lesions in the guinea pig (Krey and Silverman, 1978) produced a decline of 95% in the LHRH terminals in the median eminence. However, the same lesions had no adverse effect on the distribution of LHRH fibers in the OVLT, the suprachiasmatic nucleus or any of the other fiber tracts discussed in this study.

It should also be noted that Barry (1976c) has reported LHRH neurons within the amygdala and mesencephalon of the guinea pig. In this study we were not able to confirm the presence of these cells. False negative results remain a major problem in immunocytochemistry and our inability to demonstrate these cells could be due to many factors, such as the difference in strain or preparation of the animals, antisera used or fixation procedures.

There may be significant species differences in the distribution of both LHRH neurons and sites of termination. The hypothalamus of most species appears to have both a caudal group of LHRH neurons within the medial basal hypothalamus and a more rostral group in the medial preoptic and pericommissural region. The only exception at the present to this rule appears to be the lack of the rostral group in the hamster. LHRH neurons within the septum and around blood vessels of the anterior perforated substance have only been reported in the guinea pig as have cells in the amygdala and mesencephalon (Barry, 1976c). The num-

bers of cells in these various regions and their exact location within the various nuclear groups may vary from species to species, but this can only be determined by detailed studies and precise reporting of detailed neuroanatomical maps. The degree to which any apparent differences may be due to differences in technique or antisera can not yet be determined. It is also possible that investigators have simply not examined extrahypothalamic areas for LHRH neurons. One documentable species difference is in the regional distribution of the LHRH terminals within the median eminence. This is shown most clearly in Figs. 7A and B.

The question still exists as to the neuroendocrine role(s) of these various LHRH cell groups within the central nervous system. In the guinea pig, arcuate nucleus lesions produce a marked deficit in basal LH release in the male and severely attenuate the postcastration rise in LH release in females (Krey and Silverman, 1978). This indicates that the tubero-infundibular LHRH projection is essential for tonic LH secretion to occur in this species. This effect cannot be ascribed to destruction of the anterior pituitary gland or its blood supply since the pituitaries in the lesioned animals were free of necrosis and were able to support normal adrenal weights. Similar selective effects of arcuate nucleus lesions on tonic LH secretion have also been reported for the rat and rhesus monkey (Cheung and Davidson, 1977; Piacsek and Hofstetter, 1977; Plant et al., 1978).

Those LHRH neurons in the preoptic area which project into the median eminence do not seem to be essential for the control of tonic gonadotropin secretion. Lesions in the preoptic area or suprachiasmatic nucleus failed to disrupt the pituitary-gonadal axis in the male guinea pigs or block the postcastration rise in the female (Krey and Silverman, 1978). Further support for the hypothesis that the preoptico-infundibular LHRH network plays little role in regulating tonic LH release are the observations that hypothalamic disconnection with a Halasz-type knife, a procedure which severs these axons, fails to influence tonic gonadotropin secretion in guinea pigs or other species (Butler and Donovan, 1971; Halasz and Gorski, 1967; Krey et al., 1975).

Recently, Greeley and coworkers (1978) reported that rats with arcuate nucleus lesions induced by neonatal administration of monosodium glutamate showed a postcastration rise in gonadotropins. These authors suggest that the arcuate-median eminence tract was not essential to tonic gonadotropin secretion and, by implication, that the preoptico-infundibular tract was essential. However, one cannot rule out the possibilities that: 1) the LHRH neurons survived this treatment or 2) that those LHRH neurons in the arcuate nucleus migrated into another nuclear group in response to the treatment.

The role of the preoptic LHRH neurons in the regulation of cyclic LH release also remains unresolved. Transection of preoptico-infundibular pathways with a Halasz knife in the guinea pig fails to prevent the preovulatory surge of gonadotropins (Wiegand and Terasawa, 1978). A complete medial basal hypothalamic island in the rhesus monkey also has little effect on ovulation (Krey et al., 1975). In rat (Halasz and Gorski, 1967) and hamster (Norman et al.,

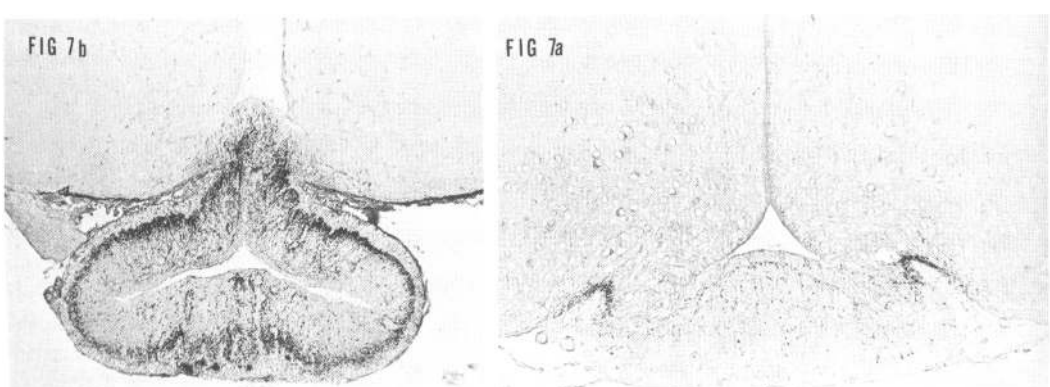


FIG. 7. LHRH axons in the median eminence of: A) the mouse and B) the guinea pig. Note the strikingly different distribution of positive axons through this structure in these two mammals.

1972), on the other hand, interruption of rostral inputs to the medial basal hypothalamus results in permanent blockade of ovulation. Whether this effect can be attributed to interruption of the preoptico-infundibular LHRH fibers or to other neuron pathways is open to question.

The role of LHRH-containing neurons which project to regions other than the median eminence is not known. These neurons may function in the regulation of sexual behavior. Systemic or intraventricular injections of exogenous LHRH do stimulate lordosis in rats (Moss and Foreman, 1976; Pfaff, 1973). LHRH neurons and terminals are present in areas shown to be involved in lordosis behavior (Gorski, 1976), as well as in sites which contain estradiol concentrating neurons (Pfaff and Keiner, 1974; Warembourg, 1977).

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