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LHRH-Systems in the Brain of the Golden Hamster

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Summary. Vibratome sections of male hamster brains were treated immunohistochemically with LHRH antiserum, and the anatomical distribution of LHRH immunoreactive cells and nerve fibers was assessed. LHRH-cell bodies are found in the ventral hypothalamus that includes its preoptic, anterior and central parts, in the septum, the olfactory tubercle, the main and accessory olfactory bulb, and the prepiriform cortex. In addition, extracerebral LHRH-neurons and ganglia exist in LHRH-positive nerves at the ventromedial surface of the olfactory tubercle and bulb as well as in olfactory nerves. Dense networks of LHRH-immunoreactive fibers are found in all regions where LHRH-cell bodies exist. Intraseptal connections reach the organum vasculosum of the lamina terminalis, the subfornical organ, and the lateral ventricle. Dorsolateral projections from the septum can be traced via the fimbria hippocampi and alveus to the ventral hippocampus, via the stria terminalis to the amygdala and piriform cortex. Ventrolateral projections extend from the level of the olfactory tubercle and preoptic-anterior hypothalamic area via the ventral amygdalofugal pathway to the prepiriform and piriform cortex as well as the amygdala. Dorsal supracallosal projections via the stria longitudinalis are seen in the induseum griseum and the cingulate cortex. Caudal efferents reach the habenula, interpeduncular nucleus, midbrain raphe, and central gray of the rostral fourth ventricle via the stria medullaris and fasciculus retroflexus and by a ventral projection via the periventricular and subventricular hypothalamus. A major portion of this ventrocaudal projection gives rise to a dense network in the median eminence. Anatomical relationships of LHRH-fibers to certain regions of the inner ventricular and outer brain surface are noted.

Key words: Hamster – LHRH – Immunohistochemistry – Olfactory systems – Feedback loops.

Anatomical identification of LHRH-systems is necessary for the understanding of LHRH-actions on the regulation of pituitary gonadotropes, reproductive behavior,

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and other functions of the endocrine and autonomic systems. Most of the immunohistochemical studies on LHRH-distribution published in the literature focus on the hypothalamus and septum. Only a few encompass the whole brain (for review see Barry 1979c). The aim of the present study is to provide an overview of the topography of LHRH-neuronal perikarya and of the circuitry of LHRH-nerve fibers in the hamster brain.

Materials and Methods

Male golden hamsters (*Mesocricetus auratus*) were castrated for 5–10 days, in order to increase the production of LHRH. In addition, three different treatments were applied: (a) colchicine (250 μg/100 g b.w.), dissolved in 50 μl isotonic saline, injected retrooculary through the superior orbital fissure 48 h before sacrifice ($n = 4$); (b) atropine sulfate (6 mg/100 g b.w.), injected s.c. 24 h before sacrifice, nialamide (75 mg/100 g b.w.) and 5-hydroxytryptophan (10 mg/100 g b.w.), both injected i.p. 6 h before sacrifice ($n = 8$); (c) 25% ethanol and 25% sucrose dissolved in distilled water, in lieu of tap drinking water, ad libitum for 4–10 weeks ($n = 4$). Also, intact untreated animals were used ($n = 4$).

Animals were killed either by decapitation, followed by immersion fixation or by cardiac perfusion with 4% paraformaldehyde and 2% formaldehyde in 0.02 M phosphate buffer-isotonic saline (PBS) containing 0.06 M sucrose. For the study of the olfactory nerves the skull was decalcified by incubation in 5% solution of EDTA (disodium-ethylenediaminetetraacetate) in phosphate buffer for 7 days prior to the immunohistochemical procedure.

Serial coronal or sagittal vibratome sections (50 and 100 μm) or paraffin sections (7 μm) were used. The vibratome sections were incubated for 48 h at 4° C with LHRH antiserum No. 44–7 (Dr. Barry) diluted 1 : 1000. Sheep anti-rabbit IgG (1 : 100; Miles Fine Chemical Co.), rabbit anti-peroxidase (1 : 100; Bionetics Co.) and peroxidase (0.5 mg/100 ml PBS; Sigma Chemical Co.) were applied each for 2 h at room temperature. All previous substances were diluted in 0.1 M PBS containing 0.02% Triton X-100. The sections were stained for 15–30 min with a solution containing 75 mg 3, 3′-diaminobenzidine-4HCl (Aldrich) and 7 μl H_2O_2 per 100 ml 0.05 M TRIS-saline buffer. The immunological specificity and properties of the LHRH antiserum 44-7 have been established (Barry 1979b). Paraffin sections were used to confirm anatomical details.

Results

Animals treated with colchicine or ethanol showed heaviest immunoreactive staining. Therefore, the mapping of the labeled structures is based mainly on results obtained from these animals. The results from untreated animals or from animals treated with atropine sulfate, nialamide, and 5-hydroxytryptophan were used in part for confirming the results obtained after colchicine or ethanol treatment. No attempts were made for quantitative comparisons concerning the effects of the different treatments. With all the treatments, perikarya and processes of certain neurons are labeled. The frequency of labeled neurons in frontal planes is depicted diagramatically in Fig. 1. Labeled neurons, in general, appear elongated and bipolar or unipolar (Fig. 2). The stained fibers resemble strings of pearls that can be followed for up to several millimeters, depending on thickness and plane of section. Erythrocytes are also stained due to endogenous peroxidase

A. Distribution of LHRH-containing Neuronal Cell Bodies

Associated with the olfactory bulb, at least four LHRH-neuronal systems can be identified, one at its medial surface within the arachnoid, one in the accessory bulb,

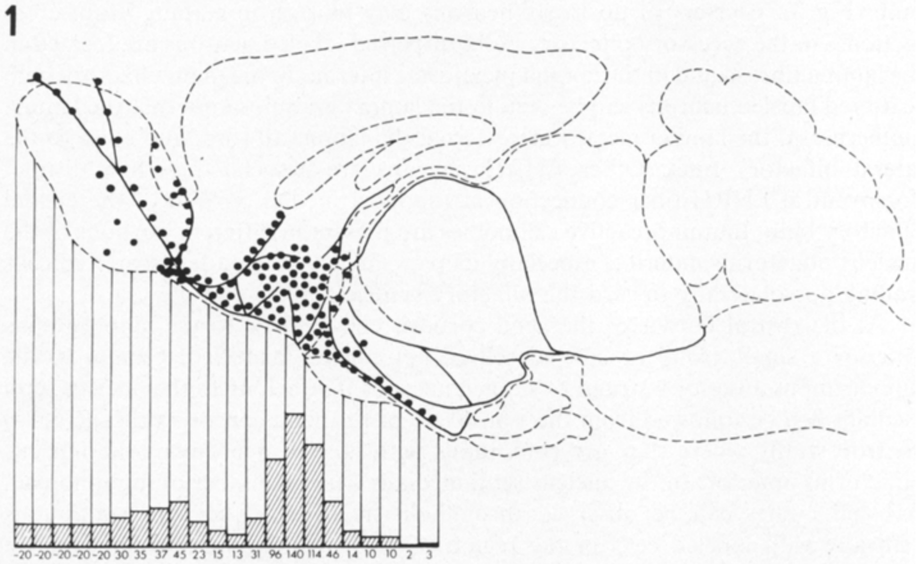


Fig. 1. Distribution of LHRH-cell-bodies im hamster brain, projected on sagittal midline plane. LHRH-containing neurons indicated by dots, major LHRH-tracts by solid lines. Scale represents number of LHRH-immunoreactive neurons in corresponding cross sections

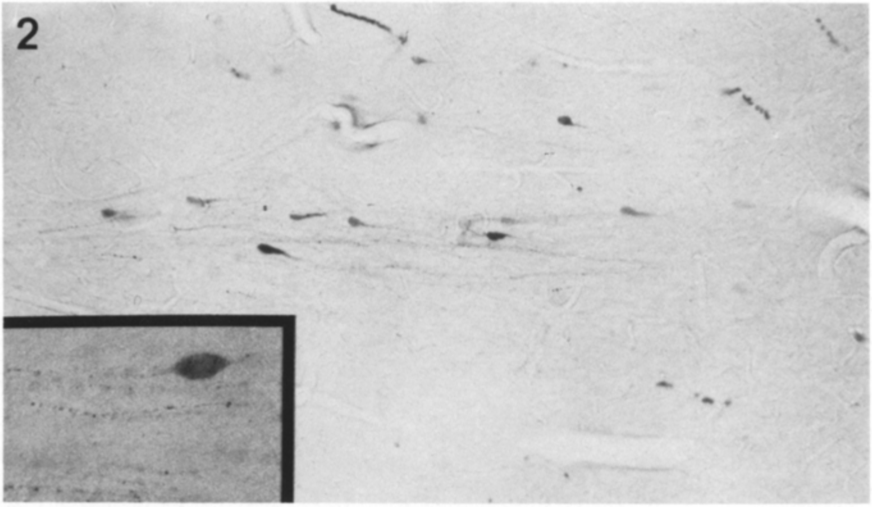


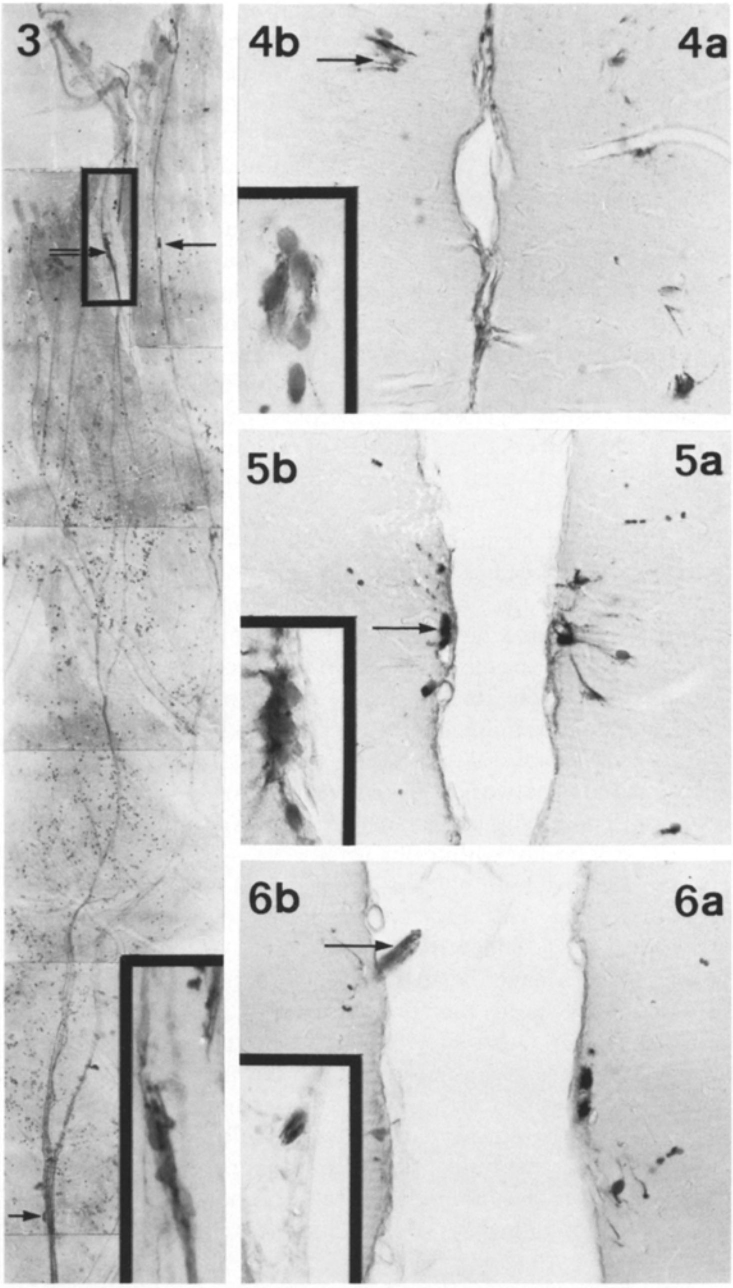
Fig. 2. LHRH-containing neurons in rostral septum adjacent to hippocampus anterior. Beginning of septo-olfactory tract. × 110. *Insert:* Bipolar LHRH-immunoreactive nerve cell body in this tract. × 265

one in the main olfactory bulb, and one in the olfactory peduncle. The extrabulbar system consists of dispersed LHRH-neurons and accumulations of neurons (ganglia), that can be followed throughout the course of the LHRH-nerve within the arachnoid of the olfactory bulb. LHRH-neurons are also observed in that portion of the LHRH-nerve that joins the olfactory nerves rostral to the olfactory bulb (Fig. 3). Clusters of up to six neurons may be seen in certain 50 µm cross sections. In the accessory olfactory bulb, dispersed labeled neurons are located in the lamina fibrosa and in the lamina plexiforme interna. In the main olfactory bulb scattered labeled neurons can be seen in the lamina granulosa interna, the lamina glomerulosa, the lamina plexiforme externa, the lamina fibrosa, and close to the lateral olfactory tract. Other LHRH neurons are associated with a distinct dorsoventral LHRH-fiber connection at the most medial portion of the caudal olfactory bulb. Immunoreactive cell bodies are present in different portions of the nucleus olfactorius anterior, especially its pars medialis with a few scattered cells trailing dorsolaterally toward the olfactory ventricle.

At the rostral border of the genu corporis callosi close to the hippocampus anterior a small group of labeled cell bodies can be identified. Caudal to the hippocampus anterior a strand of stained neurons that belong to the nucleus septi medialis can be followed from the ventral tip of the genu corporis callosi (Fig. 2) ventrorostrally where they are continuous with labeled neurons of the nucleus olfactorius anterior. In the nucleus septi medialis a large number of immunoreactive cell bodies can be observed throughout its extent. They are continuous ventrally with labeled cells in the region of the organum vasculosum laminae terminalis and the nucleus tractus diagonalis. Caudally they are linked with labeled cells in the nucleus triangularis septi and with a group of labeled cells in the ventromedial portion of the nucleus septi lateralis. Ventral to the anterior commissure stained neurons are accumulated in the periventricular region that includes the nucleus periventricularis, nucleus praeopticus pars suprachiasmatica and the nucleus praeopticus medialis. The labeled neurons in the preoptic region are continuous with labeled cells rostrally in the region of the organum vasculosum laminae terminalis and the diagonal band and caudally in the nucleus periventricularis of the anterior hypothalamus. A few scattered labeled cells are seen in the nucleus suprachiasmaticus and in the vicinity of the nucleus supraopticus, as well as in lamina 1 of the prepiriform cortex. The bed nucleus of the stria terminalis and the nucleus accumbens are essentially free, except for an occasionally stained neuron. Single labeled cells have also been noted in the ventral anterior and central hypothalamus, including the rostral nucleus arcuatus.

B. Distribution of LHRH-containing Nerve Fibers

Among the nerve fibers labeled with LHRH-antibodies, three major sites of origin may be identified, the septum-olfactory tubercle, the preoptic-anterior hypothalamus and the olfactory bulb. LHRH-fibers may be subdivided into intracerebral and extracrebral systems. As major intracerebral systems can be distinguished: rostral, dorsal, ventral, and caudal projections as well as intraseptal and intrahypothalamic connections. Certain projections appear as parallel running fibers, some of which are contained within classical pathways, such as the stria terminalis, the stria medullaris, fimbria hippocampi and fasciculus retroflexus. Other projections appear as a dense network and are less confined, such as the intraseptal and periventricular hypothalamic projections. The term tract, although frequently used here for many of the projections, does not always denote a concentrated and well confined pathway of fibers. The close relationships of many



Figs. 3–6. LHRH-nerve at medial surface of olfactory bulb (Fig. 3, sagittal section, frontal end on top with olfactory nerve) and olfactory tubercle (Figs. 4–6, cross sections, from caudal to rostral). In Figs. 4–6 condensation and formation of septo-olfactory LHRH-tract can be followed. Disperse LHRH-fibers (Fig. 4a) converge to form a tract (Fig. 4b, *arrow*) that reaches the surface (Figs. 5a, 5b *arrow*, 6a) and emerges as a nerve in the subarachnoid space (Fig. 6b, *arrow*, and *insert*). *Inserts* in Figs. 4b and 5b are higher magnifications of sections of corresponding locations with LHRH-neurons within this tract. Also note LHRH-neurons (Fig. 3, *arrow*; see also Figs. 7, 8) and ganglia (Fig. 3, *double arrow* and *insert*) in LHRH-nerve. Fig. 3: ×55; *insert*: ×190; Figs. 4–6, and *insert* Fig. 6: ×135; *inserts* Figs. 4, 5: ×270

of the projections to the ventricles and subarachnoidal space are noteworthy. Examples of immunohistochemically stained nerve fibers and tracts in different brain regions are shown in Figs. 2–26, and an overview of the projections as they may be distinguished topographically is provided by a diagram (Fig. 27).

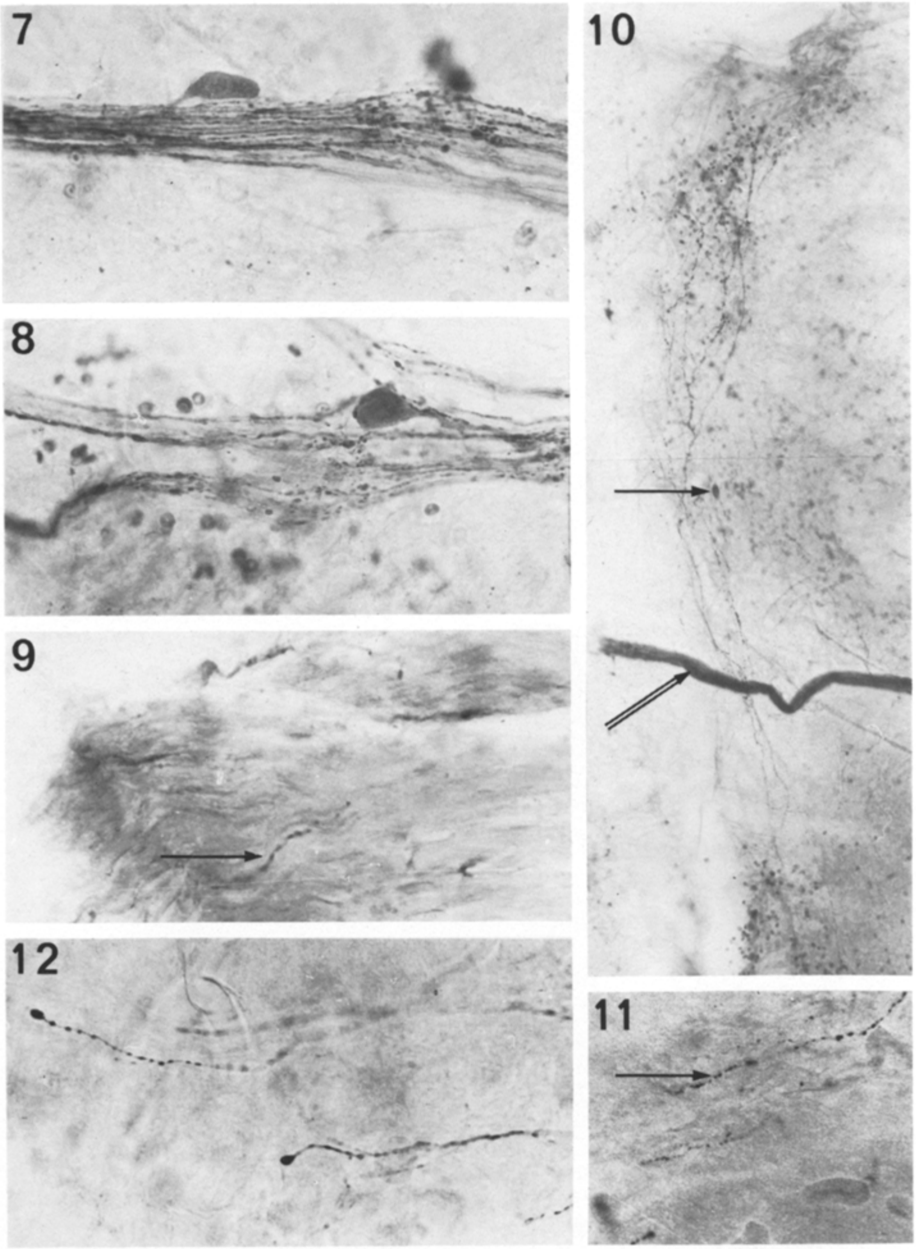
Rostral Pathways. LHRH-containing perikarya of the nucleus septi medialis immediately adjacent to the hippocampus anterior give rise to the dorsal part of the tractus septo-olfactorius. This tract can be followed from an area caudal to the hippocampus anterior passing ventrorostrally and entering the tractus olfactorius medianus where it is joined by LHRH-fibers that originate in the olfactory tubercle, the nucleus tractus diagonalis, the nucleus olfactorius anterior, and the caudal septum. During its rostral course this tract turns medially to leave the brain and to enter the subarochnoid space between the hemispheres (Figs. 4–6). There this tract is enveloped by arachnoidal tissue and resembles a peripheral nerve. Intrafascicular neurons and ganglia contribute fibers to this LHRH-immunoreactive nerve which splits several times (Figs. 3, 7, 8). At the rostral pole of the olfactory bulb, these LHRH-nerves join olfactory nerves (Figs. 3, 9).

LHRH-fibers that supply the main olfactory bulb appear to originate from neurons in the central septum, the nucleus tractus diagonalis, the olfactory tubercle, and the nucleus olfactorius anterior, pars medialis. Upon entering the olfactory bulb this tract splits into a central branch, which supplies the neck of the olfactory bulb; into a dorsal branch, which runs to the medial aspect of the accessory bulb; and into a rostral branch, which supplies the medial part of the main olfactory bulb. A heavily stained LHRH-bundle runs in the lamina plexiforme externa of the caudal olfactory bulb from ventral to dorsal or vice versa (Fig. 10). It appears to interconnect the dense LHRH-networks in the ventral main olfactory bulb (peduncle) and the accessory bulb. This connection receives an additional supply of nerve fibers from LHRH-cell bodies within this tract.

The pathway to the accessory bulb gives rise to a dorsal network within the accessory bulb and continues as a ventral tract at the border to the main olfactory bulb. The dorsal network is most evident near the entrance of the vomeronasal nerve. Here many LHRH-fibers leave the accessory bulb either entering into the vomeronasal nerve or coursing along the surface of the accessory bulb in the subarachnoid space (Figs. 11, 13). Other LHRH-fibers seem to terminate near the surface of the accessory bulb as is suggested by bouton-terminal-like distensions (Fig. 12). The accessory bulb receives also LHRH-fibers from the ventral tract before it continues rostrally in the dorsal main olfactory bulb.

At the caudal third of the olfactory bulb, fibers from the dorsoventral tract form a dense network at the ventral surface that is comparable to that one described for the accessory bulb. At this region numerous LHRH-fibers leave the brain ventrally (Fig. 14).

The main olfactory bulb receives an additional periventricular LHRH-projection. Fibers of this projection apparently originate in the central and rostral septum, forming a rostral extension of the septal subependymal tract along the medial wall of the lateral ventricle and the olfactory ventricle. In the main olfactory bulb these fibers arborize among cells of the lamina granulosa interna.



Figs. 7–12. LHRH-fibers in LHRH-nerve (Figs. 7, 8, 10), olfactory nerve (Fig. 9, *arrow*), vomeronasal nerve (Fig. 11, *arrow*), and main olfactory bulb (Fig. 12). Extracerebral LHRH-neurons in LHRH-nerve (Figs. 7, 8; Fig. 7, is higher magnification of Fig. 3, *arrow* at bottom). In Fig. 10, in addition to LHRH-nerve (*double arrow*), note dorsoventral LHRH-connection at medial surface of caudal olfactory bulb with LHRH-neuron (*arrow*). Figs. 7, 8: × 340; Figs. 9, 12: × 440; Fig. 10: × 90; Fig. 11: × 240

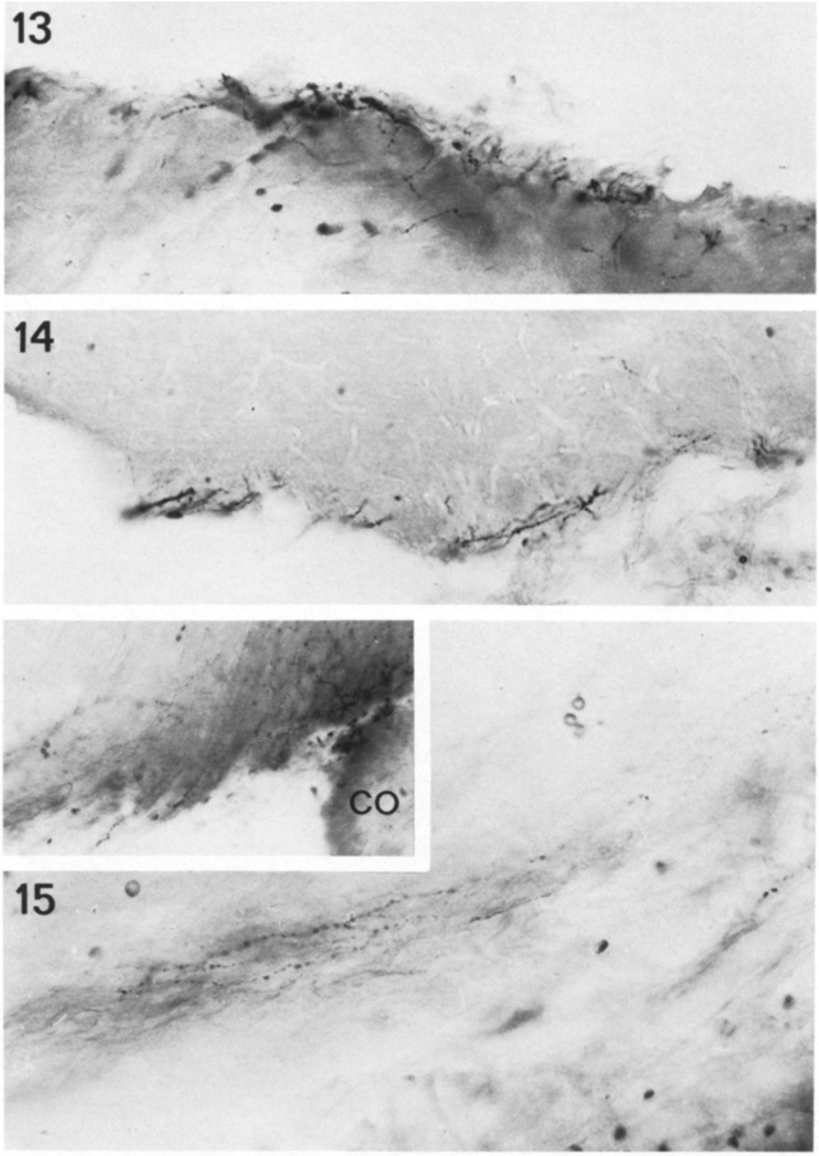
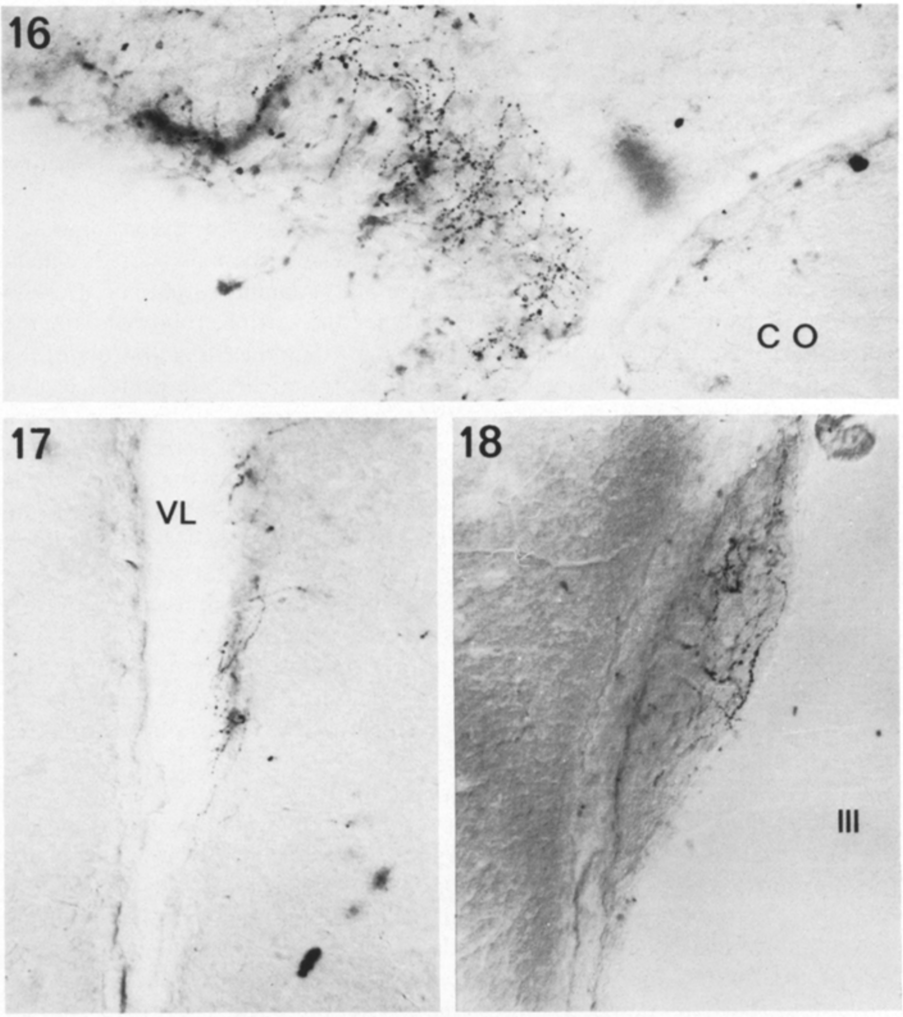


Fig. 13–15. LHRH-fibers associated with outer brain surface: at accessory olfactory bulb (**Fig. 13**), ventral caudal main olfactory bulb (**Fig. 14**), and substantia perforata (**Fig. 15**). *Insert:* low power micrograph of same region and optic chiasm (*CO*). Sagittal sections. Figs. 13, 14: × 170; Fig. 15: × 275; *Insert* Fig. 15: × 110

In the olfactory tubercle rostral to the diagonal band, numerous LHRH-fibers leave the septo-olfactory tract and course ventrally toward the substantia perforata where they form a dense network from which some fibers appear to leave the brain (Fig. 15). This portion of the olfactory tubercle also appears to receive LHRH-fibers from caudal regions.



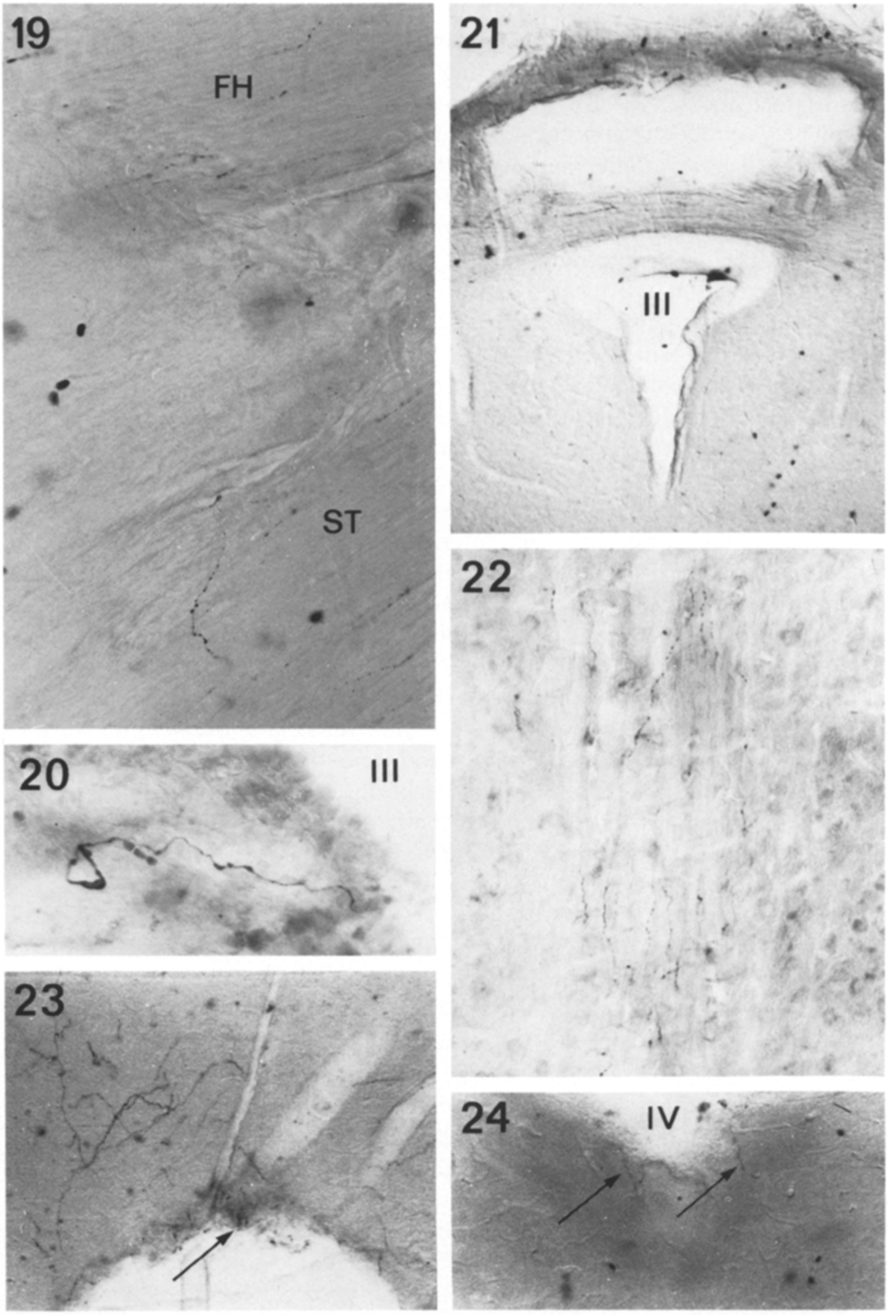
Figs. 16–18. LHRH-fibers in vascular organ of lamina terminalis (**Fig. 16**) rostral to optic chiasm (*CO*); ependyma of septal lateral ventricle (*VL*) (**Fig. 17**), and subfornical organ at third ventricle (*III*) (**Fig. 18**). Sagittal sections: Fig. 16 (× 175) and Fig. 18 (× 110); cross section: Fig. 17 (× 140)

Intraseptal Connections. Throughout the septum LHRH-fibers can be seen, however, with different densities in different regions. Since many of the immunoreactive perikarya are found in the medial and ventral septum, most of these fibers are believed to be derived from these neurons and are, therefore, called intraseptal radiation. In addition, incipient major tracts can be recognized. In the ventral septum heavy arborizations are seen in the area of the diagonal band that extend laterally into the prepiriform cortex. The highest accumulation of fibers is present at the optic recess of the third ventricle in the prechiasmatic region of the organum vasculosum laminae terminalis (Fig. 16). A portion of the fibers that reach the organum vasculosum appear to arrive from neurons in the preoptic-periventricular region. Heavily stained nerve fibers extend rostroventrally from the organum vasculosum. Many of these fibers that seem to be part of the vascular organ penetrate the brain surface.

In the rostral septum only a few fibers enter the nucleus accumbens which otherwise appears to be free of immunoreactive material. Similarly, in the midseptal region, the bed nucleus of the stria terminalis contains only sparse fibers. While numerous LHRH-fibers are observed in close proximity to the commissura anterior, in the commissure itself only a few occasional fibers are noted, which, however, do not follow the mediolateral course of the commissure, in contrast to some of the pericommissural fibers. In the midline, fibers can be followed from the organum vasculosum dorsally that form a network along the rostral border of the third ventricle toward the nucleus triangularis septi and the subfornical organ. From this midline region of the central septum a distinct band of divergent fibers extends to the ventral half of the lateral ventricle. This tract is formed in part by fibers that arrive from the nucleus of the diagonal band and pass the n. accumbens at its medial border. Other fibers from the midseptum contribute to this tract. The main portion of this intraseptal lateral ventricular projection can be followed along the ependyma where it forms a subependymal plexus. Some of these fibers traverse the ependyma (Fig. 17). In the dorsolateral part of the supracommissural septum a less intense network of fibers exists, compared to its ventrolateral part. The dorsal septum receives fibers from the intraseptal dorsal midline projection which enter the fimbria hippocampi and contribute to the dense network in the subfornical organ. There, some of the fibers can be followed to the surface of this circumventricular organ (Fig. 18).

Lateral Projections. The lateral projections may be subdivided into a hippocampal, a dorsal amygdaloid, and a ventral amygdaloid-piriform pathway. The septo-hippocampal tract can be followed in the commissura fornicis ventralis where it is in juxtaposition to the third ventricle and, more caudally, in the ventral part of the fimbria hippocampi (Fig. 19). Further caudally LHRH-fibers remain in the ventral aspect of the fimbria hippocampi where they split and enter into the ventral hippocampus. The lateral branch follows the alveus and the medial branch courses at the medial border of the hippocampus. Sparse LHRH-fibers are found throughout the different regions of the hippocampus inferior including the dentate gyrus and subiculum. In the dorsal hippocampus no LHRH-fibers are seen.

The origin of the dorsal septo-amygdaloid tract can be traced to a region of the septum that lies immediately ventral to the origin of the septo-hippocampal tract. While the fibers of the septo-hippocampal tract converge at the medial aspect of the lateral ventricle, the fibers of the dorsal septo-amygdaloid tract converge at the ventrolateral aspect of the lateral ventricle. From there the dorsal septo-amygdaloid tract can be followed within the stria terminalis (Fig. 19), first in its dorsal part, and, further caudally, along the medial surface of the amygdaloid complex. In the amygdala, LHRH-fibers arborize and course toward the nucleus medialis and corticalis, with a few fibers in the nucleus centralis. Sparse LHRH-fibers can also be detected in the outer layers of the piriform cortex with highest concentration in the transitional zone to the nucleus corticalis. In the caudal



Figs. 19–24. LHRH-fibers in fimbria hippocampi (*FH*) and stria terminalis (*ST*) (**Fig. 19**); choroid plexus of third ventricle (*III*) (**Fig. 20**); posterior and habenular commissures (**Fig. 21**); fasciculus retroflexus (**Fig. 22**); interpeduncular nucleus (**Fig. 23**), and fovea centralis of fourth ventricle (*IV*) (**Fig. 24**). *Arrows* point at surface relationships. Cross sections. Fig. 19: × 240; Fig. 20: × 170; Fig. 21: × 70; Fig. 22: × 180; Fig. 23: × 110; Fig. 24: × 90

amygdala, LHRH-fibers are seen dorsally along the lateral wall of the lateral ventricle in the region of the nucleus basalis and lateralis. Caudal to this level, LHRH-fibers in the amygdala that belong to the stria terminalis are parallel and close to LHRH-fibers in the inferior hippocampus that belong to the alveus. At the transition between the subiculum and the caudal nucleus medialis of the amygdala, LHRH-fibers of both origins merge and their derivation cannot be identified.

Ventrolateral projections to the telencephalon from the tuberculum olfactorium, the septum, and the preoptic-anterior hypothalamic region probably follow the ventral amygdalofugal pathway. LHRH-fibers that are oriented in a mediolateral direction are located close to the base of the olfactory tubercle, septum, and hypothalamus, and extend laterally into lamina I of the prepiriform and piriform cortex. There, the most lateral regions contain only sparse fibers compared to the more medial regions. Some of these fibers are within the tractus olfactorius lateralis, while most of them course dorsally to it. Some fibers leave this ventrolateral LHRH-tract dorsally into the prepiriform and piriform cortex, the amygdala and the lateral hypothalamus. In the midline LHRH-fibers course in the supraoptic commissures of Ganser and Meynert. A few of these fibers traverse the region of the supraoptic nucleus laterally. More caudally, in the lateral hypothalamus, LHRH-fibers course dorsally and laterally around the optic tract. As the optic tract assumes a more dorsal position, some LHRH-fibers traverse the optic tract at its dorsal pole to pass laterally toward the amygdaloid complex.

Dorsal Projections. Immunoreactive fibers are seen in the trunk of the corpus collosum near the septal region. These sparse fibers are oriented in various directions, most frequently rostrocaudally, and seem to enter the indusium griseum and the stria longitudinalis. LHRH-fibers can be observed throughout the course of the stria longitudinalis from the genu to the level of the splenium of the corpus callosum. A few fibers, apparently derived from this projection, are noted in deeper layers of the overlying cingulate cortex.

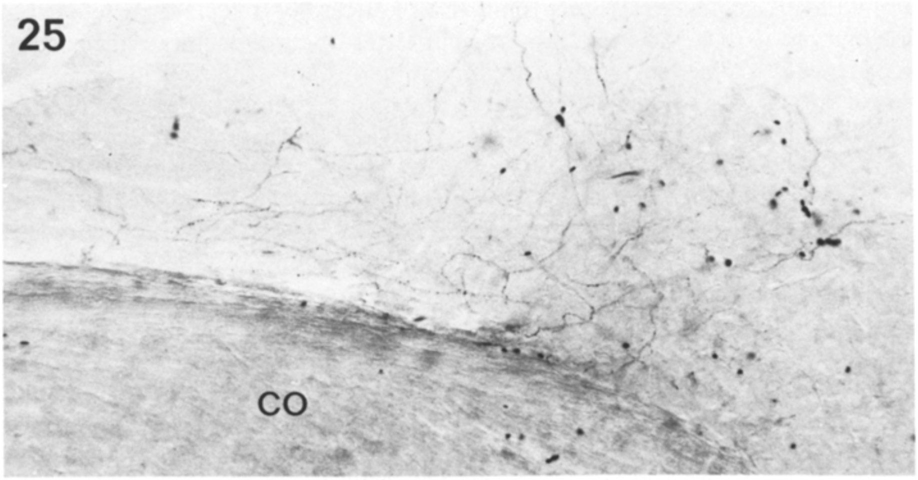
Caudal Projections. The caudal projection of LHRH-fibers that are derived from the septum follow first the stria medullaris and then the fasciculus retroflexus. It can be traced to the interpeduncular nucleus of the midbrain, the pontine raphe nuclei and the central gray of the fourth ventricle at the fovea centralis and locus caeruleus.

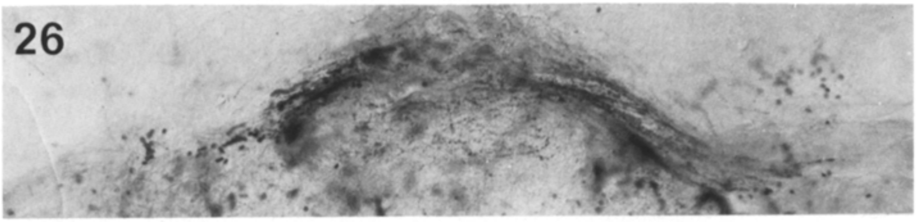
LHRH-positive fibers that course in the stria medullaris appear to originate close to those of the stria terminalis and can be traced to the ventrolateral region of the lateral ventricle in the septum. When the stria medullaris reaches the habenular region, immunoreactive fibers are most numerous in the medial part of the tract and within the medial habenular nucleus. At this level a few fibers leave dorsally toward the choroid plexus (Fig. 20). LHRH-fibers are coursing horizontally within the habenular commissure and in the posterior commissure. The ependyma of the subcommissural organ appears to be free of LHRH-fibers. Numerous LHRH-fibers surround the pineal recess, including the region of the lamina intercalaris. Some of these fibers penetrate the ependyma and can be traced to the surface of the ventricular lumen (Fig. 21). A few fibers occur in the superior colliculus.

In the fasciculus retroflexus, numerous LHRH-fibers have no preferential distribution (Fig. 22). From this tract LHRH-fibers enter the nucleus interpeduncularis. The most intense accumulation of LHRH-fibers is in the ventral part of this nucleus, where LHRH-fibers from the fasciculus retroflexus merge with those coming from hypothalamic periventricular and subventricular projections. In the interpeduncular recess LHRH-fibers exist close to the surface, some of which seem to penetrate the surface of the brain and to enter the subarachnoidal space (Fig. 23). Other LHRH-fibers are continuing caudally along the ventral border of the interpeduncular nucleus and turn dorsally and dorsocaudally toward the nucleus raphe magnus and dorsalis as well as the central gray of the pons. There fibers are noted in the region of the fovea centralis, the medial eminence, and the locus caeruleus. Some of these LHRH-fibers appear to penetrate the ependyma to enter the fourth ventricle (Fig. 24). Caudal to this level no LHRH-fibers can be seen.

Septo-hypothalamic and Hypothalamo-fugal Projections. In all regions of the hypothalamus LHRH-fibers can be detected, with highest concentrations in the median eminence, followed by the periventricular ventral hypothalamus. The latter includes the preoptic region, the anterior hypothalamic area, and the central and posterior hypothalamus. Because of the dense arborization in these regions, the origin of projections is difficult to determine. Apparently, fibers from LHRH-immunoreactive neurons in the septum course together with fibers from LHRH-immunoreactive neurons in the preoptic and ventral anterior hypothalamic regions to give rise to a ventral subventricular tract and a less confined periventricular projection. Fibers of the subventricular tract can be seen rostrally in the suprachiasmatic region above the optic chiasm coursing in part within the suprachiasmatic commissures of Ganser and Meynert (Fig. 25), and caudally, immediately below the surface of the tuber cinereum where they give rise to the dense arborizations in the median eminence (Fig. 26). Caudal to the median eminence subventricular fibers can be followed below the surface of the mammillary body to reach the interpeduncular recess. Throughout the course of the subventricular projection, fibers leave this tract and run in a dorsal and also lateral direction toward the lateral hypothalamus. In the preoptic hypothalamic area these fibers continue laterally into the medial and ventral amygdala and piriform cortex following the ventral amygdalofugal pathway.

Throughout the periventricular hypothalamus, LHRH-fibers branch in a centrifugal direction and arborize at varying degrees in hypothalamic nuclei, including the periventricular, paraventricular, ventromedial, dorsomedial, and arcuate nuclei as well as the lateral and posterior hypothalamus. Periventricular fibers can be traced toward the zona incerta and the dorsocaudal thalamic extent of the third ventricle. LHRH-fibers of the periventricular network give rise to a supramammillary tract at the supramammillary commissure that continues into the interpeduncular region. Nuclei of the mammillary body show few LHRH-fibers. At the rostral level of the interpeduncular nucleus, the fibers from the subventricular and periventricular projections merge. Some of these fibers arborize in the nucleus interpeduncularis, while others merge with fibers from the dorsal septo-mesencephalic projection.





Figs. 25 and 26. LHRH-fibers in anterior hypothalamic region dorsal to optic chiasm (*CO*) (Fig. 25, sagittal section) and caudal median eminence (Fig. 26, cross section). × 110

Fig. 27. Schematic representation of LHRH-fiber-systems in hamster brain. LHRH-fibers projected to midline. Major pathways indicated by thick solid line. Hippocampal and amygdaloid LHRH-fiber-systems at bottom left. *HI* hippocampus, *ST* stria terminalis, *FH* fimbria hippocampi, *am* amygdala

In addition to these caudal projections, rostral hypothalamic projections originate from LHRH-cell bodies in the rostral ventral hypothalamus. From these neurons fibers course in rostral and dorsal directions, merging with fibers that are apparently derived from septal LHRH-neurons.

Discussion

The results of this study indicate the presence of most LHRH-producing neurons in the septal-preoptic region with a smaller number rostrally in the olfactory tubercle, the olfactory bulb, and caudally in the anterior and ventromedial hypothalamic area. This agrees well, regarding the septo-hypothalamic regions, with observations made in other rodents such as rat, mouse, and guinea pig (Barry et al. 1973; Baker et al. 1975; Silverman and Krey 1978; Jennes and Croix 1980). In rabbit, cat, and dog, as well as in squirrel monkey and human, the number of LHRH perikarya in the ventromedial hypothalamus appears to be more numerous than in rodents (for review see: Barry 1979a, c; Silverman et al. 1979).

In the male hamster many LHRH-projections from the septo-preoptic region exist in classical efferent pathways, such as the fimbria hippocampi, stria terminalis, stria medullaris, fasciculus retroflexus, ventral amygdalofugal pathway, lateral olfactory tract, posterior commissure, habenular commissure, stria longitudinalis, and medial forebrain bundle. Also, there is considerable congruency between the results from this immunohistochemical study and those obtained with anterograde transport of amino acids (Meibach and Siegel 1977; Swanson and Cowan 1979). Since many of these pathways and sites of projection are known to influence LHRH-secretion, it is suggested that certain of these LHRH-projections form an intracerebral feedback system (Fig. 28). This probably includes LHRH-fibers that project to the raphe nuclei in order to influence serotonin secretion and to neurons of the olfactory system in order to modulate sensory inputs. The wide distribution of LHRH-projections argues not only for effects on gonadotropin secretion and mating behavior (Moss et al. 1979; Riskind and Moss 1979), but for other modes of autonomic-endocrine regulation. Modulation of the firing rate after iontophoretic

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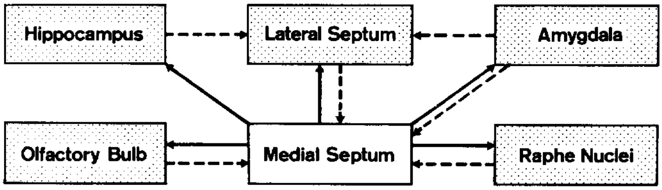

Fig. 28. LHRH-feedback loops between medial septum and other brain regions as postulated from anatomical evidence (selected examples). LHRH-pathways (*solid lines*) project to regions that are known to send efferents (*dashed lines*) to medial septum where many LHRH-cell bodies are located. Most LHRH-projection sites are target regions for sex steroid hormones (*dotted areas*). Other similar feedback loops are likely to exist

application of LHRH supports the view that this peptide acts not only as a neurohormone but also as a neurotransmitter or modulator (Renaud et al. 1976; Richard 1978).

A comparison of the distribution of LHRH-producing neurons with target neurons for estrogen in the rat and guinea pig has led to the proposal that some or all LHRH-neurons are targets for estradiol (Stumpf and Sar 1977). The present data, if compared with the distribution of estrogen-target-cells in other rodents and in the hamster (unpublished), suggest that to a certain extent the population of LHRH-producing cell bodies does not overlap the distribution of estradiol-target-neurons. This includes especially LHRH-neurons in the nucleus septi medialis, where no estrogen-target-neurons have been located. Some LHRH-neurons and estrogen-target-cells may be identical, for example, those in the diagonal band, ventrolateral dorsal septum and medial preoptic area. A combination of autoradiography and immunohistochemistry in the same preparation as demonstrated for ^{3}H estradiol and neurophysin (Sar and Stumpf 1980) would be required to decide whether or not certain LHRH-neurons are targets for estradiol.

Sites of projection of LHRH-pathways agree with certain sites of accumulation of estradiol-concentrating neurons as well as target neurons for androgen and progesterone. Thus, there appears to be considerable congruency between many of the LHRH-fiber projections and the "estrogen-neuron circuitry" introduced earlier (Stumpf 1970). According to the results of autoradiographic studies, the stria terminalis has been implicated as the major "sex pathway" (Stumpf 1968, 1975). These anatomical data suggest that LHRH-projections directly influence estrogen-target-neurons and that, on the other hand, estrogen influences LHRH-neurons indirectly and/or directly via suggested intracerebral feedback loops.

It is obvious that the anatomical distribution of LHRH-cell bodies in the rostral hypothalamus and septum challenges the concept of the "hypophysiotrophic area" as the sole site of origin of LHRH (Szentagothai et al. 1972). The inappropriateness of this concept had been pointed out repeatedly on the grounds of the anatomical distribution of steroid-hormone target neurons (Stumpf and Sar 1973).

An important observation is the existence of LHRH-neurons, ganglia, and nerve fibers *outside* of the brain. This extracerebral LHRH-system resembles anatomically the peripheral autonomic nervous system.

Olfactory and vomeronasal nerves are accompanied by LHRH-fibers that originate either in the olfactory bulbs or in more caudal telencephalic neurons or both. The fact that LHRH-fibers leave the olfactory bulb through the main and accessory nerves suggests an effect of LHRH on olfactory receptor cells in the nasal mucosa. This is further supported by the finding that many LHRH-fibers seem to course and terminate in the subarachnoid space without entering the olfactory nerve. As there is a direct connection of the subarachnoid space of the olfactory bulb with the nasal mucosa (Warwick and Williams 1973), it seems probable that LHRH is able to reach this target also by diffusion through the cerebrospinal fluid.

The presence of LHRH-fibers in the olfactory bulb of the hamster has been reported by Hoffman et al. (1979) who observed immunoreactive fibers in the glomerulose, granulose, and plexiforme layer. The results of the present study agree with results published in the literature, according to which the olfactory system exerts strong effects on behavior and regulation of the estrous cycle (Whitten 1958; Winans and Powers 1977; Horton and Shepherd 1979; Murphey and Schneider

1970). This effect may be due to olfactory output as well as LHRH input that modulates olfactory response. The olfactory bulb receives LHRH-afferents from the septal-olfactory tubercle region and the olfactory bulb sends efferents (DeOlmos 1972; Broadwell 1975; Heimer 1975; Scalia and Winans 1975; Davis et al. 1978) directly and indirectly, via the piriform cortex and amygdala, to the septum and olfactory tubercle. This provides another example for the proposed existence of intracerebral feedback loops.

LHRH-fibers leave the brain not only through the main and accessory olfactory bulbs, but reach the subarachnoidal space at several other sites which include the substantia perforata and the interpeduncular recess. It is noteworthy that these regions of the brain are covered by arachnoidal cisterns. In a manner similar to their penetration to the outer surface of the brain, LHRH-fibers can be seen in certain regions of the ventricular system to course below the ependyma and to penetrate it, entering the ventricular cerebrospinal fluid. These regions include the septal lateral ventricle, the subfornical organ, the ventral portion of the third ventricle, the lamina intercalaris, and the ventral fourth ventricle. In addition, a few fibers are associated with the choroid plexus of the third ventricle. These observations together suggest that LHRH is released not only at certain pericapillary or neuronal sites within the brain but also at the ventricular and the subarachnoidal liquor space. Because of such anatomical relationships and because LHRH has been assayed in the cerebrospinal fluid (Joseph et al. 1975) where enzymatic activity is low (Davison 1970; Vigneri et al. 1979), the cerebrospinal fluid probably is a route of transport for LHRH and perhaps other messengers to certain cerebral and extracerebral sites of action.

References

Baker BL, Dermody WC, Reel JR (1975) Distribution of gonadotropin-releasing hormone in the rat brain as observed with immunocytochemistry. Endocrinology 97:125–135

Barry J (1979a) The distribution, organization and connections of neurons containing luteinizing hormone releasing hormone in the vertebrate brain. Coll Int CNRS 280:399–413

Barry J (1979b) Extra-hypophyseal immunoreactive LH-RH pathways in primates. Proc Int Symp Neuroendocrinol Regulatory Mech, pp 13–25

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

Barry J, Dubois MP, Poulain P (1973) LRF producing cells of the mammalian hypothalamus. Z Zellforsch 146:351–366

Broadwell RD (1975) Olfactory relationships of the telencephalon and diencephalon in the rabbit. I. An autoradiographic study of the efferent connections of the main and accessory olfactory bulbs. J Comp Neurol 163:329–346

Davis BJ, Macrides F, Youngs WM, Schneider SP, Rosene DL (1978) Efferents and centrifugal afferents of the main and accessory olfactory bulbs in the hamster. Brain Res Bull 3:59–72

Davison H (1970) Physiology of the cerebrospinal fluid. J A Churchill, London

DeOlmos JS (1972) The amygdaloid projection field in the rat as studied with cupricsilver method. In: BE Eleftheriou (ed), Advances in Behavioral Biology, Vol. 2, The Neurobiology of the Amygdala. Plenum Press, New York, pp 145–204

Heimer L (1975) Olfactory projections to the diencephalon. In: WE Stumpf and LD Grant (eds), Anatomical Neuroendocrinology. Int Conf Neurobiology of CNS-hormone Interactions. Karger Verlag, Basel, pp 30–39

Hoffman GE, Davis BJ, Macrides F (1979) LHRH perikarya send axons to the olfactory bulb in the hamster. 9th Proc Soc Neurosci Abstracts 1779

Horton LW, Shepherd BA (1979) Effects of olfactory bulb ablation on estrus-induction and frequency of pregnancy. Physiol Behav 22:847–850

Jennes L, Croix D (1980) Changes in the LHRH-immunoreactivity of hypothalamic structures during late pregnancy and after parturition in the guinea pig. Cell Tissue Res 205:121–131

Joseph SA, Sorrentino S, Sundberg DK (1975) Releasing hormones, LRF and TRF, in the cerebrospinal fluid of the third ventricle. In: Knigge KM, Scott DE, Kobayashi H, Ishii S (eds) Brain-Endocrine Interaction II. The Ventricular System. Karger Verlag, Basel, pp 306–312

Meibach RC, Siegel A (1977) Efferent connections of the septal area in the rat: An analysis utilizing retrograde and anterograde transport methods. Brain Res 119:1–20

Moss RL, Riskind P, Dudley CA (1979) Effects of LHRH on sexual activities in animal and man. In: Collu et al. (eds) Central Nervous System Effects of Hypothalamic Hormones and Other Peptides. Raven Press, New York, pp 345–366

Murphy MR, Schneider GE (1970) Olfactory bulb removal eliminates mating behavior in the male golden hamster. Science 167:302–303

Renaud LP, Martin JB, Brazeau P (1976) Hypothalamic releasing factors: Physiological evidence for a regulatory action on central neurons and pathways for their distribution in brain. Pharmacol Biochem Behav 5:171–178

Richard Ph, Freund-Mercier MJ, Moos F (1978) Les neurones hypothalamiques ayant une fonction endocrine. J Physiol, Paris 74:61–112

Riskind P, Moss RL (1979) Midbrain central gray: LHRH infusion enhances lordotic behavior in estrogen-primed ovariectomized rats. Brain Res Bull 4:203–205

Sar M, Stumpf WE (1980) Simultaneous localization of ^{3}H estradiol and neurophysin 1 or arginine vasopressin in hypothalamic neurons demonstrated by a combined technique of dry-mount autoradiography and immunohistochemistry. Neurosci Lett (in press)

Scalia F, Winans SS (1975) The differential projections of the olfactory bulb and accessory olfactory bulb in mammals. J Comp Neurol 161:31–56

Silverman AJ, Krey LC (1978) The luteinizing hormone-releasing hormone (LH-RH) neuronal networks of the guinea pig brain. I. Intra- and extrahypothalamic projections. Brain Res 157:233–246

Silverman AJ, Krey LC, Zimmerman EA (1979) A comparative study of the luteinizing hormone releasing hormone (LHRH) neuronal networks in animals. Biol Reprod 20:98–110

Stumpf WE (1968) Estradiol-concentrating neurons: Topography in the hypothalamus by dry-mount autoradiography. Science 162:1001–1003

Stumpf WE (1970) Estrogen-neurons and estrogen-neuron systems in the periventricular brain. Am J Anat 129:207–218

Stumpf WE (1975) The brain: An endocrine gland and hormone target. In: Stumpf WE, Grant LD (eds) Anatomical Neuroendocrinology. Int Conf Neurobiology of CNS-Hormone Interactions. Karger Verlag, Basel, pp 2–8

Stumpf WE, Sar M (1973) Hormonal inputs to releasing factor cells, feedback sites. Progr Brain Res 39:53–71

Stumpf WE, Sar M (1977) Steroid hormone target cells in the periventricular brain: relationship to peptide hormone producing cells. Fed Proc 36:1973–1977

Swanson LW, Cowan WM (1979) The connections of the septal region in the rat. J Comp Neurol 186:621–656

Szentágothai J, Flerkó B, Mess B, Halász B (1972) Hypothalamic control of the Anterior Pituitary. Adadémiai Kiadó, Budapest

Vigneri R, Filetti S, Pezzino V, Squatrito S, Polosa P, Scapagnini U (1979) Hypothalamus-pituitary connections: The possible role of cerebrospinal fluid in the transmission of the hypophysiotrophic signal. In: Polleri A, MacLeod RM (eds) Neuroendocrinology: Biological and Clinical Aspects. Academic Press, London, pp 67–76

Warwick R, Williams PL (1973) Gray's Anatomy, WB Saunders, Co, Philadelphia, pp 990

Whitten WK (1958) Modification of the oestrous cycle of the mouse by external stimuli associated with the male. J Endocrinol 17:307–313

Winans SS, Powers JB (1977) Olfactory and vomeronasal deafferentation of male hamsters: Histological and behavioral analysis. Brain Res 126:325–344

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