The effects of epithalamic transection (ET) on pineal biochemistry at 06.30 h (30 min before light onset)

Treatment		NAT pmol/organ·h	HIOMT pmol/organ·h	Melatonin pg/organ
C	(n = 5)	481.1 ± 74.6	61.3 ± 6.2	362.3 ± 138.8
Sh	(n = 4)	460.1 ± 82.4	60.1 ± 5.3	370.2 ± 82.1
ETc	(n = 8)	601.2 ± 70.1	69.5 ± 5.1	873.5 ± 325.4
ETi	(n = 6)	621.1 ± 48.7	68.4 ± 6.4	400.6 ± 21.0

Results are expressed as means ± SD. C, Control animals; Sh, Sham-operated animals; ETc, Animals receiving a complete epithalamic transection; ETi, Animals receiving an incomplete epithalamic transection.

In a recent anterograde tracing study[7], projections from the PVN to the pineal have been demonstrated to be located in the stria medullaris thalami. This observation inspired the present study in which the striae medullaris were cut bilaterally to examine what effects this might have on pineal melatonin synthesis. The results obtained show a clear decrease of NAT and HIOMT activities at night (= 3.00 h). These effects were not merely the consequence of a disturbed blood flow in the vicinity of the pineal, since no changes in pineal function were noted in sham-operated animals which had received only sagittal sinus-transection. However, in groups with lesions of the hippocampus and certain neocortical areas only, a decrease in the activity of both enzymes was also observed which suggests that the hippocampal formation itself or other efferent fibers influence pineal biochemistry. As shown by electrophysiology, the hippocampus is multisynaptically connected to the pineal gland[5]. Furthermore, hippocampal projections via septum and stria medullaris thalami terminate in the habenular nuclei[19] which in turn project to the pineal gland.

The role of the hypothalamus for pineal function has been investigated previously. HIOMT regulation was abolished following lateral hypothalamic lesions[20]. The circadian rhythmicity of pineal melatonin synthesis was disrupted by lesions of the hypothalamic paraventricular area[15] and electrical stimulation of the PVN resulted in a decrease of pineal NAT activity and melatonin content[16].

Whether the reduction in pineal NAT and HIOMT following epithalamic transection is the result of an overall attenuation of pineal metabolic activity or of a phase shift of the melatonin synthesis rhythm, cannot yet be resolved. The observation that pineal enzymes and melatonin content in operated animals remained elevated at the end of the dark period (table) speaks in favor of a phase shift of the circadian rhythm of melatonin synthesis.

That epithalamic inputs do influence the pineal's capacity to synthesize melatonin is an important new insight into the nature of brain-pineal interrelationships. On the basis of electron microscopic studies in the rat pineal[21], in which it was reported that axo-axonic synapses are occasionally present, it may be speculated that epithalamic neurons may serve to amplify in a tonic fashion the sympathetic neural activity in the pineal, or alternatively, that epithalamic neurons may serve a synergistic role in the control of melatonin synthesis by pinealocytes. In either case, without the presence of epithalamic inputs, pineal biochemical activity is altered. Parenthetically, it is interesting to note that lesions of the stria medullaris result in a decline of choline-acetyltransferase activity in the rat habenular nuclei[19] which are known to project afferent fibers to the pineal gland[22].

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Immunoreactive luteinizing hormone-containing neurons in the brain of the white-footed mouse, *Peromyscus leucopus*

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Summary. The distribution of immunoreactive LH in the brain of the white-footed mouse *(Peromyscus leucopus)* was determined using immunocytochemical procedures. Immunoreactive fibers are located in the hypothalamus, preoptic area, septum and amygdala. Stained cell bodies are seen in the arcuate nucleus and preoptic area. Gonadectomy enhances staining for LH in the brain.
Key words. Luteinizing hormone; immunocytochemistry; brain; white-footed mouse.

Anterior pituitary-like gonadotrophic hormones have been detected in the brain by means of radioimmunoassay and immunocytochemistry[2]. The presence of luteinizing hormone (LH) in the hypothalamus, limbic and striatal regions of rat brain[2-5] is thought to underlie a 'short' loop feedback mechanism whereby the hormone regulates its own secretion[6]. Changes in firing of

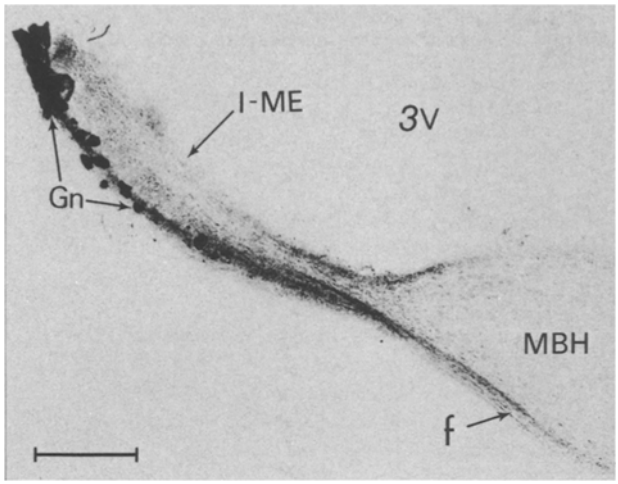
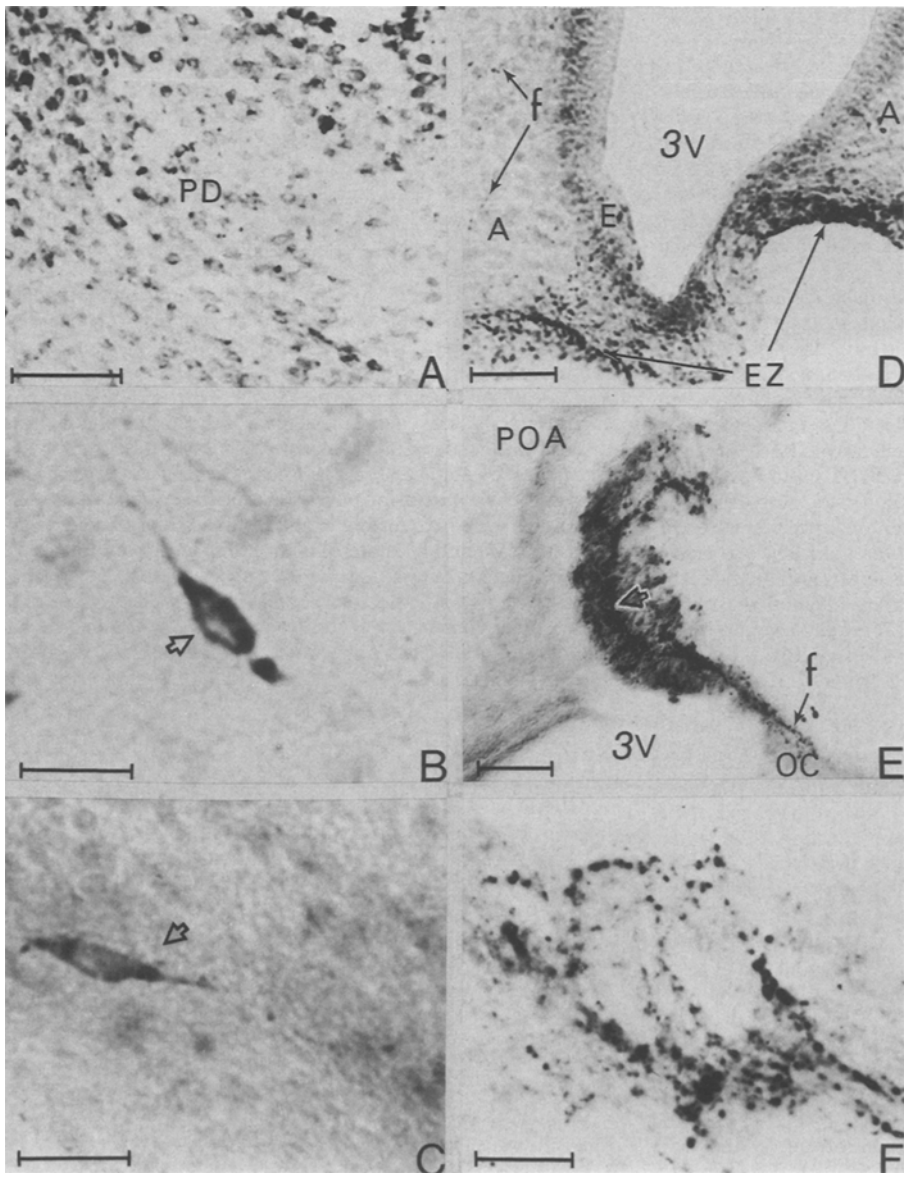


Figure 1. Parasagittal section of the ME-infundibular stalk showing LH-like immunoreactivity in gonadotrophs in the pars tuberalis and nerve fibers in the ventral region of the mediobasal hypothalamus. Some of the fibers are associated with the gonadotrophs. Bar 150 µm.

central neurons in response to administration of LH[7] indicates that LH may act as a neuromodulator to help regulate reproductive functions. At present, there is a paucity of detailed information on the cytoarchitecture of LH-containing neuronal systems in the brain. This probably is due to relatively low concentrations of immunoreactive LH in CNS neurons. In the present study, immunocytochemical demonstration of LH in the brain was conducted in animals in which the quantity of LH in the brain was increased by gonadectomy. Removal of endogenous sex steroids markedly increased the immunoreactivity of LH in brain, and enhanced visualization of LH-containing neurons.

Materials and methods. Sexually mature female white-footed mice *(Peromyscus leucopus)* were sacrificed 4 weeks after bilateral ovariectomy, and their brains and pituitary glands were processed for immunocytochemical demonstration of LH. Details of immunocytochemical procedures are provided in a previous study[8]. Brain sections were cut at 100 µm on a vibratome (Lancer). Consecutive sagittal sections were taken 2 mm lateral from the midline medially to the 3rd ventricle. For one animal, consecutive coronal sections were taken from the rostral preoptic area (POA) to the mammillary bodies. Pituitaries were embedded in gelatin, and vibratome sections were cut at 20 µm. Sections were treated for the demonstration of LH using the



Anatomical abbreviations in figures

A	arcuate nucleus
E	ependyma
EZ	external zone of the median eminence
f	immunoreactive fiber
I-ME	infundibular stalk-median eminence
MBH	mediobasal hypothalamus
OC	optic chiasma
PD	pars distalis
POA	medial preoptic area
3V	third ventricle

Figure 2. *A* Gonadotrophs in the pars distalis. Bar 50 µm. *B* and *C* Immunoreactive cell bodies (arrows) in the arcuate nucleus and medial POA, respectively. Bars 20 µm. *D* Coronal section of the ME showing immunoreactive fibers in the external zone, arcuate nucleus and ependymal lining of the third ventricle. Bar 40 µm. *E* Parasagittal section of the OVLT showing the dense plexus of immunoreactive fibers. Intense staining is present in a band of fibers near the capillary network of the organ (arrow). Bar 50 µm. *F* Parasagittal section of the triangular nucleus of the septum that contains a major plexus of immunoreactive fibers and granules that contain reaction product. Bar 20 µm.

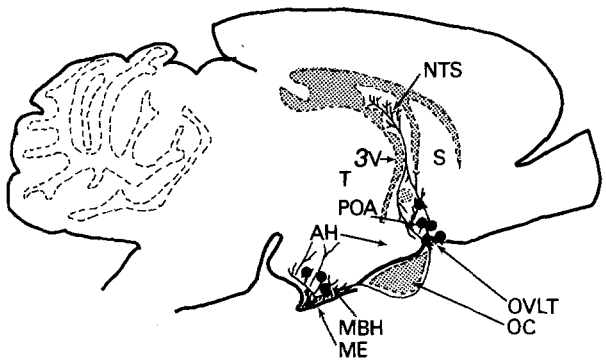


Figure 3. Diagrammatic parasagittal section of the brain of *P. leucopus* showing the distribution of immunoreactive cell bodies (●) and fibers (thin lines).

Anatomical abbreviations in figures

A	arcuate nucleus
AH	anterior hypothalamic area
E	ependyma
EZ	external zone of the median eminence
f	immunoreactive fiber
I-ME	infundibular stalk-median eminence
MBH	mediobasal hypothalamus
ME	median eminence
NTS	triangular nucleus of the septum
OC	optic chiasma
OVLT	organum vasculosum of the lamina terminalis
PD	pars distalis
POA	medial preoptic area
S	septum
T	thalamus
3V	third ventricle

NIAMDD-anti-hLH beta IC-1 rabbit antiserum. This antiserum is specific for hLH when tested in vitro against hLH, hFSH, and hTSH, and preabsorbtion of the primary antiserum with rat LH inhibits staining for LH in the pituitary and brain.

Sections were incubated with H_2O_2 to inactivate endogenous peroxidase and incubated with primary antiserum diluted 1/400 in Tris buffered saline (containing 0.1% Triton X-100) for 48 h at 4°C. Sections sequentially were incubated with second antibody (sheep antirabbit gamma globulin serum), rabbit peroxidase-antiperoxidase complex (Sigma) and reacted with 3,3-diaminobenzidine tetrahydrochloride and H_2O_2. Sections were floated onto glass slides, dehydrated and mounted with coverslides using Permount. Staining for LH was abolished when incubation buffer was substituted for primary antiserum, sheep antirabbit gamma globulin serum or the peroxidase-antiperoxidase complex.

Results. Within the pituitary gland, LH-like immunoreactivity is present in gonadotrophs of the pars distalis and in the pars tuberalis, adherant to the median eminence (ME)-infundibular stalk (figs 1, 2A). The pars intermedia and pars nervosa are not stained. Immunoreactive fibers in the brain have a densely-staining, beaded appearance, similar to that of peptidergic neurons. Conspicuous fiber plexuses are present in the ME, organum vasculosum of the lamina terminalis (OVLT) and the triangular nucleus of the septum (NTS; fig. 2D, E, F). Some fibers in these regions contain large deposits of immunoreactive material. Diffuse networks of immunoreactive fibers are located in the medial POA, medial septum, amygdala and arcuate nucleus. Also, large numbers of specifically-stained fibers are associated with the ependymal lining of the 3rd ventricle. A major compact preoptic-infundibular pathway of LH fibers (located in the ventral hypothalamus) extends from the ME to the OVLT and medial POA. Some fibers of this pathway course ventral to the optic chiasma. Within the ME-infundibular stalk region, many labeled fibers of this pathway appeared to be associated with immunostained gonadotrophs in the pars tuberalis (fig. 1). A second, more diffuse, pathway exists between the medial POA and the NTS. Cell bodies that contain reaction product are bipolar neurons that stained from dark brown to black, with the chromogen deposited throughout the cytoplasm. The nucleus is free of reaction product. Average dimensions of the cell bodies are 9 × 20 μm. Immunoreactive cell bodies are most abundant in the medial and lateral POA. Labeled cell bodies also are present in the arcuate nucleus and the lateral posterior hypothalamus (fig. 3).

Discussion. The results from this study confirm and extend those of previous studies on the distribution of LH-like immunoreactivity in rat brain[2]. The majority of immunoreactive fibers are situated within the hypothalamus, medial POA, septum and amygdala. In *P. leucopus*, the LH-immunoreactive fibers are located near the cell bodies of neurons that produce gonadotropin-releasing hormone[8]. Thus, the possibility exists that LH may help regulate its own release via direct action on the GnRH neurons. The presence of LH fibers in the septum and amygdala supports the idea that LH may be involved in controlling reproductive behaviors as well as the secretion of gonadotropins. The source of immunoreactive LH in CNS neurons is speculative, but there is evidence that LH may arise from pituitary as well as from neuronal sources. For example, uptake of peripherally-administered gonadotropins by brain neurons[9], and suppression of radioimmunoassayable LH in the hypothalamus by hypophysectomy[3] indicate that LH in the brain may originate from the pituitary. The observation that immunostained fibers in *P. leucopus* are associated with LH-containing gonadotrophs in the pars tuberalis is evidence that LH may be taken up by these fibers and transported by retrograde axoplasmic flow to a variety of brain sites. Also, the intense immunoreactivity of nerve fibers in the OVLT indicates that LH may enter the brain from permeable blood vessels in this circumventricular organ[10]. The ventricular system also may be involved in the distribution of LH within the brain, because numerous immunoreactive fibers are associated with the ependymal lining of the 3rd ventricle. The recent finding that colchicine increases hypothalamic LH[5] is evidence that some LH may be produced in the brain.

The enhanced staining for LH in the brain after ovariectomy may be a consequence of increased synthesis of LH in the absence of the negative feedback action of gonadal steroids. Studies on the effect of sex steroids on brain LH-immunoreactive neurons are in progress.

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