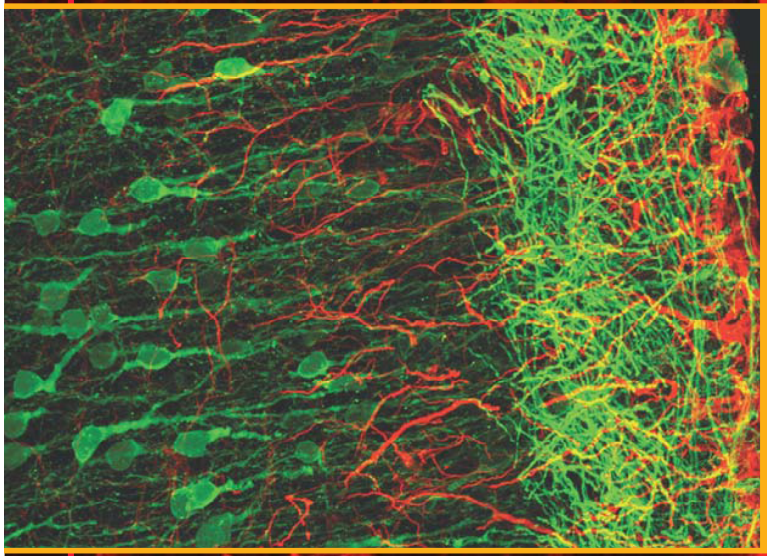
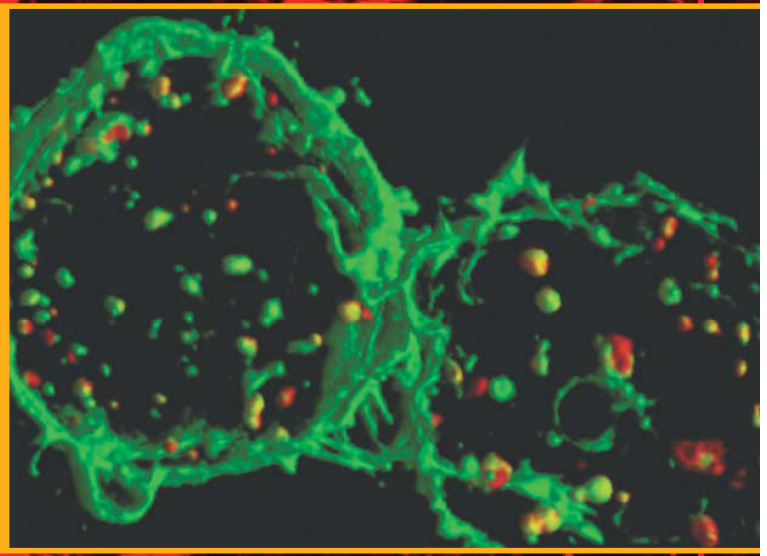
Neuroscience in Medicine

Edited by **P. Michael Conn**





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15 The Hypothalamus

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1. INTRODUCTION

1.1. Functions of the Hypothalamus

The hypothalamus is part of the limbic portion of the brain in vertebrates, which regulates the *internal milieu* of the cells within narrow limits as it compensates for changing external conditions, such as variations in temperature, energy, or defensive requirements. The constancy of the internal environment resulting from these fine adjustments made by the hypothalamus is known as *homeostasis*. The hypothalamus maintains homeostasis by exerting control over the two regulatory systems of the organism: the *nervous system* and the *endocrine system*.

In regulating the nervous system, the hypothalamus controls *autonomic processes*, such as cardiovascular, thermoregulatory, and visceral function. In addition, *behavioral processes* that include ingestive, sexual, maternal, and emotional behaviors are also regulated by the hypothalamus. The role of the hypothalamus in the regulation of the nervous system is summarized in Table 1.

In its regulation of the function of the endocrine system, the hypothalamus exerts control of the two subdivisions of the *pituitary gland*. The *anterior pituitary* or *adenohypophysis* synthesizes hormones that regulate adrenal, thyroid, and gonadal function as well as growth and lactation. The synthesis and secretion of anterior pituitary hormones, in turn, are regulated by peptides and amines. These are synthesized by and secreted from specific hypothalamic neurons, and are transported to the adenohypophysis through a microscopic vascular route, known as the *hypothalamo–hypophyseal portal system* (Fig. 1) to either stimulate or inhibit the synthesis and secretion of specific hormones of the adenohypophysis. These peptides and amines are known collectively as *releasing* or *release-inhibiting hormones* (Table 2).

The hormones of the *posterior pituitary* or *neurohypophysis* are synthesized by specific hypothalamic neurons and transported to the neurohypophysis axonally by the *hypothalamo–hypophyseal tract* (Fig. 1), released into sinusoids and ultimately into the peripheral circulation to directly regulate blood pressure, water balance, and milk ejection (Table 2).

The fact that certain neurons can subserve two functions—the receipt and transmission of electrical information as typical nerve cells and as endocrine cells that secrete their products into a minute blood supply to regulate the adenohypophysis, or into the neurohypophysis and ultimately into the peripheral circulation to regulate visceral processes—led to the concept of *neurosecretion* and ultimately to the birth of the science of *neuroendocrinology*.

It is important to note that hypothalamic control of any one process is not exerted in a manner that is exclusive of other processes. Thus, the hypothalamus

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Table 1
Neural Processes Regulated by the Hypothalamus

Category	*System or activity*	*Process*
Autonomic Process	Cardiovascular	Blood flow (↓ or ↑) vasodilation or vasoconstriction
	Thermoregulatory	Blood flow, shivering, panting
	Visceral	Digestive acid secretion (↑)
Behavioral Process	Sexual	Sexual receptivity ("heat")
	Maternal	Nest building
	Emotional	Aggression (↑)
	Ingestive	Eating and drinking (↑ or ↓)

↑ = increase; ↓ = decrease.

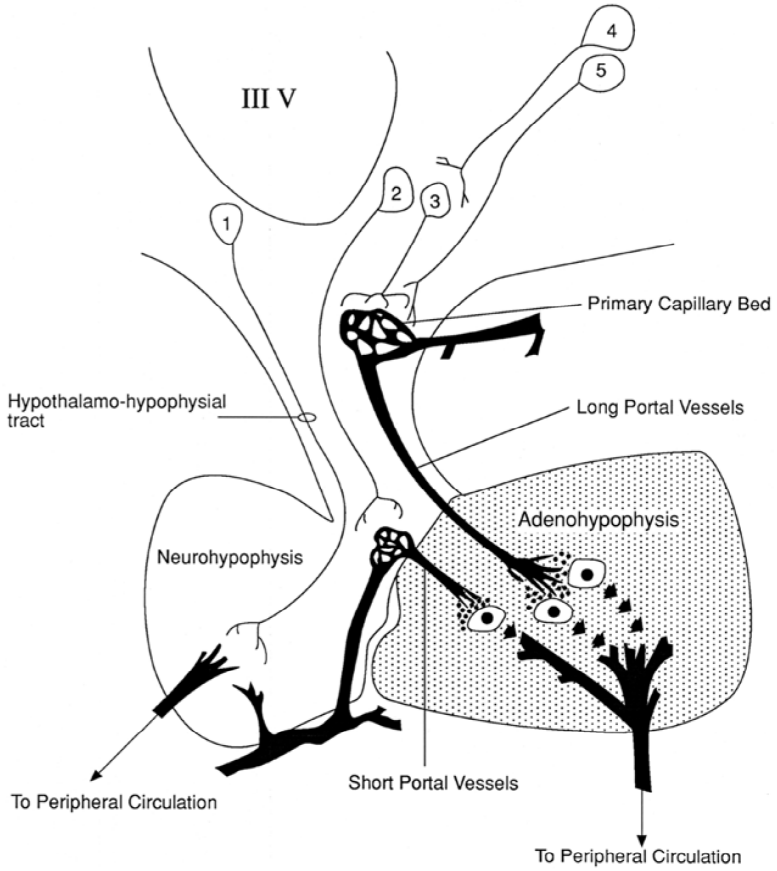

Fig. 1. Diagrammatic representation of the control of the neurohypophysis and adenohypophysis by the hypothalamus. *Neuron 1* is a peptidergic magnocellular neuron from the supraoptic or paraventricular nuclei of the hypothalamus that secretes oxytocin or vasopressin into sinusoids in the neurohypophysis. The axons of these two nuclei travel to the neurohypophysis in the hypothalamo–hypophyseal tract. *Neuron 2* could be a hypophysiotropic peptidergic or aminergic neuron terminating adjacent to the short portal vessels, which represent a potential route of communication between the neurohypophysis and adenohypophysis. The hypothalamic release or release-inhibiting peptidergic neurons are of this type. The tuberohypophysial dopaminergic neurons are also of this type. *Neuron 3* could also be a hypophysiotropic peptidergic or aminergic neuron. In this case, the neuron terminates on the primary capillary bed of the median eminence (ME). It also secretes release or release-inhibiting peptides into portal blood, which reach the adenohypophysis via the long portal vessels. The tuberoinfundibular dopaminergic neurons are also of this type.

Table 2
Neuroendocrine Regulators of Hypothalamic Origin

System regulated	*Site of regulation*	*Action*	*Hypothalamic regulator*
Thyroid gland	Adenohypophysis	Stimulation of thyrotrophin secretion	Thyrotrophin-releasing hormone
Adrenal cortex	Adenohypophysis	Stimulation of adrenocorticotrophin secretion	Corticotrophin-releasing hormone
Gonads	Adenohypophysis	Stimulation of luteinizing hormone and follicle-stimulating hormone secretion	Gonadotrophin-releasing hormone
Muscle, bone, liver	Adenohypophysis	Stimulation or inhibition of growth hormone secretion	Growth hormone-releasing hormone, somatostatin
Milk synthesis and secretion from the mammary gland	Adenohypophysis	Inhibition of prolactin secretion	Dopamine
Cardiovascular, Renal muscle, renal tubule	Vascular smooth water reabsorption	Vasoconstriction, (antidiuretic hormone)	Vasopressin
Mammary Gland, Uterus of mammary ducts and uterus	Smooth muscle pressure inducing milk ejection; increase uterine contraction in labor	Increase intramammary	Oxytocin

exerts *integrative function* over physiological processes. For example, thermoregulatory processes are governed by both the autonomic nervous system and the endocrine system. Exposure to extremes of temperature results in the adjustment of blood flow through autonomic processes and metabolic adjustments through regulation of thyroid hormone secretion. Both of these seemingly unrelated controls are under the influence of the hypothalamus.

1.2. Historical Perspective

Although there are indications that the ancients may have appreciated the vital role of higher centers in normal physiology, the role of the hypothalamus did not begin to crystallize until a series of clinical observations made from the late nineteenth to the early twentieth centuries. Most of the early studies focused on hypothalamic control of pituitary function because pituitary pathologies were the most overt. A connection between the hypothalamus and pituitary gland was not appreciated at that time; thus, many of the early observations were often mistakenly attributed directly to "pituitary tumors." In 1901, Dr. Alfred Fröhlich, a Viennese physician, correctly reported a case of adiposogenital dystrophy in a 14-yr-old boy suffering from a pituitary tumor that compressed the optic tract and hypothalamus, which was subsequently relieved by surgery. Shortly thereafter, Erdheim described gonadal atrophy and obesity directly caused by hypothalamic damage without damage of the pituitary gland. Camus and Roussay (1913) later demonstrated polyuria in dogs bearing surgical lesions of the hypothalamus without damage to the pituitary gland. These were the first direct observations that the hypothalamus controls the pituitary gland. The development of a parapharyngeal procedure to surgically remove the

(*Fig. 1. continued*) Neurons with cell bodies that lie within the arcuate and periventricular nuclei and terminate on the primary capillary bed in the ME comprise the infundibular tract. The link between the rest of the brain and the pituitary gland is represented by *neurons 4 and 5*. These neurons secrete catecholamines (and in some cases peptides) that act as neurotransmitters or neuromodulators on the hypophysiotropic neurons. The termination of neuron 4 is axo-dendritic or axo-somatic, and that of neuron 5 is axo-axonic.

pituitary gland of rats (hypophysectomy) by Philip Smith (1926) led to a flurry of studies of the pituitary gland and brain. It was appreciated at that time that the pituitary gland must remain intact with the brain for coitus to induce ovulation in rabbits. The classical, yet, crude experiments of Marshall and Verney (1936) demonstrating ovulation-induction in rabbits by passage of an electrical current through the brain were soon followed by the experiments of Geoffrey Harris (1937), which showed that more localized stimulation of the hypothalamus also led to ovulation induction in rabbits. Subsequent studies revealed that coitus would not result in ovulation in the rabbit if the pituitary stalk was cut and a foil barrier was placed between the hypothalamus and pituitary with the (mistaken) intention of preventing the regrowth of severed "nerves" (Westman and Jacobsohn, 1937). A "fast forward toward the future" allows us to determine that coitus stimulated the release of the decapeptide gonadotropin-releasing hormone (GnRH) into portal blood, and its role was to stimulate the release of an ovulation-inducing amount of luteinizing hormone (LH) into the peripheral circulation.

Perhaps the most significant early contribution to the science of neuroendocrinology was the development of the concept of *neurosecretion* by the husband-and-wife team of Ernst and Berta Scharrer. Beginning in the early 1930s, they proposed that the cells of the hypothalamus must have a unique function distinct from other brain cells based on their multinucleated appearance, the abundance of protein-containing colloid-like vacuoles, and the unique proximity between these cells and the surrounding capillary network. The Scharrers proposed that these nerve cells must therefore have a glandular function. At about the same time, Popa and Fielding (1930) described the vascular connection between the hypothalamus and adenohypophysis in rabbits, although they mistakenly believed that the direction of blood flow was from the gland toward the hypothalamus. The first report of flow toward the pituitary was made in a study of toads by Houssay (1935). The developing concept of neurosecretion coupled with the description of the portal vasculature opened the door to an innovative series of experiments demonstrating that the hypothalamus controlled the adenohypophysis with messages transported over a vascular route. However, experiments involving transection of the stalk connecting the hypothalamus with the pituitary gland led to varying results, leading some to doubt the importance of a vascular connection until Green and Harris (1947) suggested that the cut portal vessels could regenerate and Harris (1950) subsequently showed that reproductive function was restored to a degree proportional to portal vessel regeneration in stalk-sectioned rats. Finally, the elegant experiments of Harris and Jacobsohn (1952) convincingly demonstrated the primacy of the hypophyseal-portal vasculature in anterior pituitary function. In these experiments, rats were hypophysectomized, and adenohypophyses from their newborns were transplanted to either the temporal lobe of the brain or, by a transtemporal route, immediately beneath the median eminence (ME) of the hypothalamus. Only those animals with transplants beneath the ME that had been re-vascularized by the portal vasculature showed a resumption of reproductive function. In support of this, Nikitovich-Weiner and Everett (1958) autografted anterior pituitaries to the kidney capsule and demonstrated a loss of thyrod-stimulating hormone, or thyrotropin (TSH); adrenocorticotropic hormone (ACTH); follicle-stimulating hormone (FSH); and LH secretion, but an enhancement of prolactin secretion from the transplants. These transplants were subsequently removed and placed under the temporal lobe of the brain or beneath the ME. Only those rats bearing transplants to the ME showed a resumption of normal anterior pituitary function.

The dawning of the science of neuroendocrinology was completed with the focus on the chemical nature of the activities of the adenohypophysis and neurohypophysis. After the studies of Van Dyke and associates (1941) established the existence of separate oxytocic and pressor principals, du Vigneaud identified the structure of oxytocin (OT) (1950) and then vasopressin (1954). This was followed by a multitude of studies between the mid 1960s through the 1970s by Andrew Schally and Roger Guillemin's laboratories on the chemical nature of the hypothalamic neuropeptides, which control the secretion of TSH, LH/FSH, ACTH, and growth hormone from the anterior pituitary. The "arrival" of the science of neuroendocrinology was recognized by the Nobel Prizes awarded to these two investigators in 1977.

2. ANATOMY OF THE HYPOTHALAMUS

2.1. The Boundaries of the Hypothalamus are Distinctly Defined

The hypothalamus is situated in the lowermost portion of the *diencephalon* (Figs. 2 and 3). The human hypothalamus presents well-defined boundaries. The rostral border is limited by a vertical line drawn

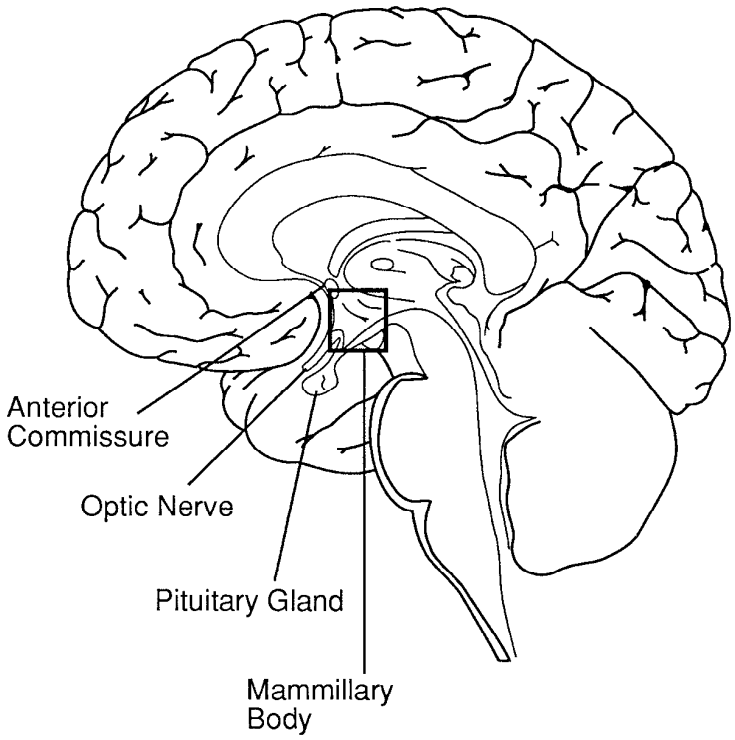

Fig. 2. The position of the hypothalamus and pituitary of the human relative to the rest of the brain. The hypothalamus is bounded by the *dark-bordered box*.

through the anterior border of the *anterior commissure*, *lamina terminalis*, and *optic chiasm*. The hypothalamus is bordered caudally by a vertical line drawn through the posterior border of the *mammillary body* as it bounds the *interpeduncular fossa*. The superior border of the hypothalamus is the *hypothalamic sulcus* as it borders the *thalamus*, and the inferior boundary is the bulging *tuber cinereum*, which tapers to form the *infundibulum*, the funnel-shaped functional connection between the hypothalamus and the pituitary gland. Laterally, the borders are poorly defined as a result of the blending of the hypothalamic gray matter with adjacent structures. However, by convention, the lateral borders are confined by the *internal capsule* and its caudal limits.

2.2. The Divisions of the Hypothalamus are Described as Functional Groupings

The hypothalamus is divided into clusters of perikarya embedded in gray matter. These are known as *nuclei* (singular: *nucleus*). Several problems are inherent in this designation. In most cases, the nuclei are not morphologically distinct structures with boundaries that are distinct in histological preparation. The dendrites and axons of these neurons may extend for distances beyond the limits of the nucleus. Moreover, chemically and functionally, the nuclei may be heterogeneous to varying degrees. Thus, only vague functional and anatomical boundaries can be drawn for hypothalamic nuclei.

The groupings of the nuclei may be described in a *rostro-caudal* direction as (i) *anterior* or *supraoptic* (Fig. 4), located between the lamina terminalis and the posterior edge of the optic chiasm; (ii) *medial* or *tuberal* (Fig. 5), located between the optic chiasm and the mammillary bodies; and (iii) *posterior* or *mammillary* (Fig 6), including the mammillary bodies and the structures just dorsal to them. The hypothalamus can also be described longitudinally in mediolateral zones (Figs. 4–6) known as *periventricular*, bordering the third ventricle; *medial*, comprising the major hypothalamic nuclei, which are sites of limbic-system projections; and *lateral*, which is separated from the medial zone by the *fornix*, a large C-shaped tract that interconnects limbic-system structures.

The most anterior hypothalamic areas are poorly defined. Rather than being grouped as diencephalic structures, the *medial preoptic* and *septal* areas are

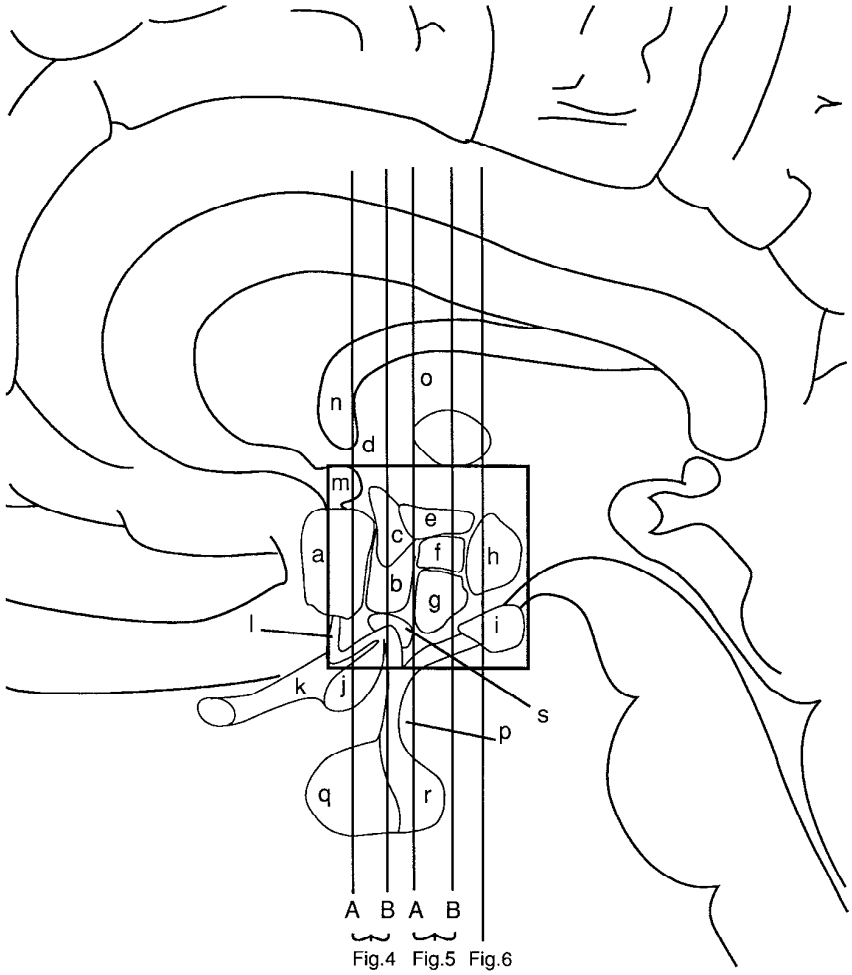

Fig. 3. The position of hypothalamic nuclei and adjacent structures in sagittal section. The vertical lines represent the planes of frontal Figs. 4A,B; 5A,B; and 6. The box outlines the corresponding area of Fig. 2. *a*, preoptic nucleus; *b*, anterior hypothalamic area; *c*, paraventricular nucleus; *d*, hypothalamic sulcus; *e*, dorsal hypothalamic area; *f*, dorsomedial nucleus; *g*, ventromedial nucleus; posterior hypothalamic area; *i*, mamillary body; *j*, optic chiasm; *k*, optic nerve; *l*, lamina terminalis; *m*, anterior commissure; *n*, fornix; *o*, thalamus; *p*, infundibulum; *q*, adenohypophysis; *r*, neurohypophysis; *s*, suprachiasmatic nucleus.

actually part of the telencephalon (Fig. 4A). However, modern embryology has shown that the preoptic area has the same embryonic origins as many diencephalic structures. Thus, the preoptic area is often considered to be part of the hypothalamus. The *medial preoptic area* (Fig. 4A, part *g*) has been shown to possess sexually dimorphic features. As described later, uniquely stained groups of neurons form in this area in organisms exposed to testosterone prenatally or neonatally. The *lateral preoptic area* (Fig. 4A, part *f*) is not morphologically distinct from the medial preoptic area, but subserves uniquely distinct physiological roles. More caudally, the *anterior* and *lateral hypothalamic areas* appear (Fig. 4B, parts *d*,*c*). The cells of these areas are small, with few dendritic branches. The lateral hypothalamic area receives fibers from the medial forebrain bundle. Chemical lesion of this area leads to aphagia. As discussed later, this area plays a stimulatory role in feeding behavior. The *paraventricular nucleus* (Fig. 5A, part *f*) is wedge-shaped, and as its name implies, lies adjacent to the third ventricle. The deeply staining neurons are of two types: *magnocellular*, or neurons with large perikarya, and *parvicellular*, or neurons with small perikarya. The axons of the magnocellular neurons terminate in the neurohypophysis, and the axons of the parvicellular neurons terminate on the primary capillary bed of the hypophyseal portal vasculature in the ME. The *supraoptic*

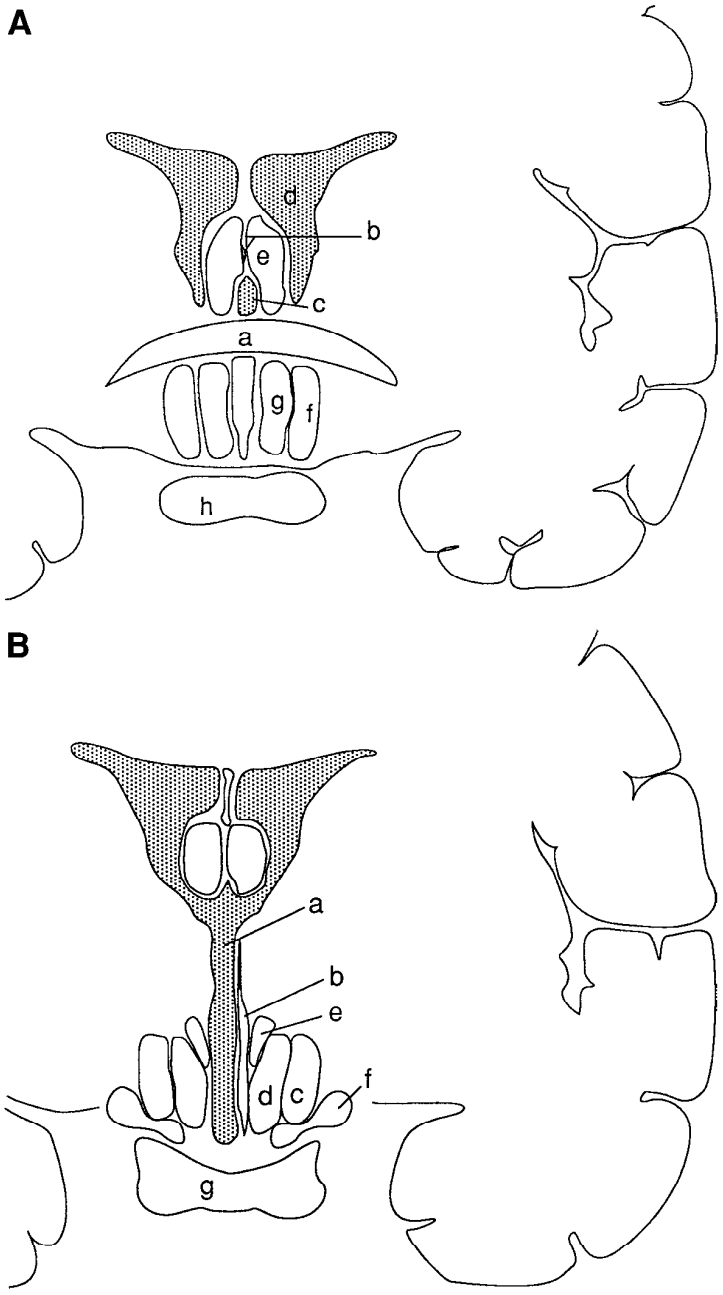

Fig. 4. The hypothalamic and adjacent structures of the anterior or supraoptic groupings. **(A)** *a*, anterior commissure; *b*, septal area; *c*, third ventricle; *d*, lateral ventricle; *e*, column of fornix; *f*, lateral preoptic area; *g*, medial preoptic area; *h*, optic chiasm. **(B)** *a*, third ventricle; *b*, periventricular nucleus; *c*, lateral hypothalamic area; *d*, anterior hypothalamic area; *e*, paraventricular nucleus; *f*, supraoptic nucleus; *g*, optic chiasm.

nucleus (Fig. 5A, part *j*; Fig. 5B, part *h*) is located directly above the beginning of the optic tracts, and consists of a large anterolateral subnucleus and a smaller posteromedial subnucleus connected by a thin strand of cells (Fig. 5A). As in the paraventricular nucleus, the neurons of the supraoptic nucleus stains darkly and consists of magnocellular perikarya with axons that terminate in the neurohypophysis. The axons of the supraoptic and paraventricular nuclei travel in a bundle, known as the *hypothalamo–hypo-*

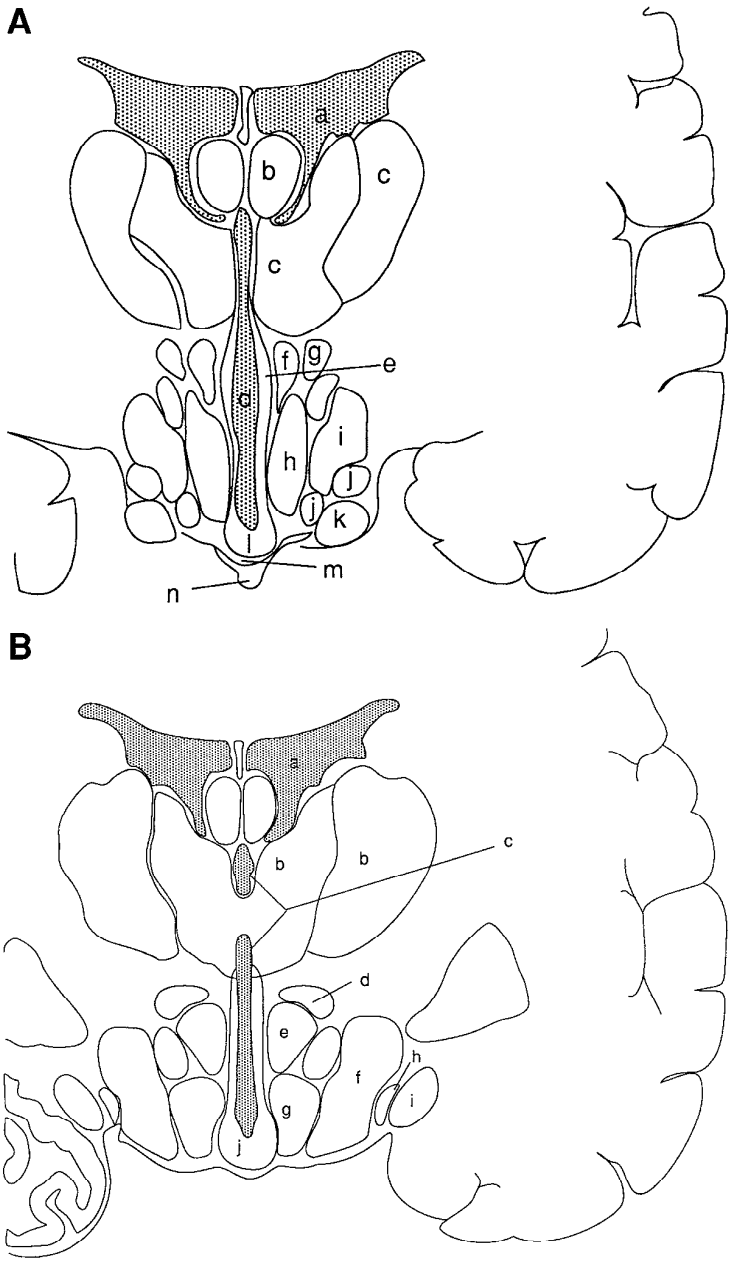

Fig. 5. The hypothalamic and adjacent structures of the medial or tuberal groupings. **(A)** *a*, lateral ventricle; *b*, body of fornix; *c*, thalamus; *d*, third ventricle; *e*, periventricular nucleus; *f*, paraventricular nucleus; *g*, dorsal hypothalamic area; *h*, anterior hypothalamic area; *i*, lateral hypothalamic area; *j*, supraoptic nucleus; *k*, optic tract; *l*, arcuate nucleus; *m*, median eminence; *n*, infundibulum. **(B)** *a*, lateral ventricle; *b*, thalamus; *c*, third ventricle; *d*, dorsal hypothalamic area; *e*, dorsomedial nucleus; *f*, lateral nucleus; *g*, ventromedial nucleus; *h*, supraoptic nucleus; *i*, optic tract; *j*, arcuate nucleus.

physeal tract, to the neurohypophysis (Fig. 1). The magnocellular and parvicellular cells of these regions produce vasopressin and OT. The *suprachiasmatic nuclei* (Fig. 3, part *s*) are distinctly staining paired structures (in rodents) overlying the *optic chiasm* (Fig. 3, part *j*). In humans, the suprachiasmatic nuclei are not strikingly distinct morphologically. In all mammals, the cells of this area receive retinohypothalamic input, and are believed to be the "circadian clock" that controls the temperature cycle, sleep/wake cycle, and

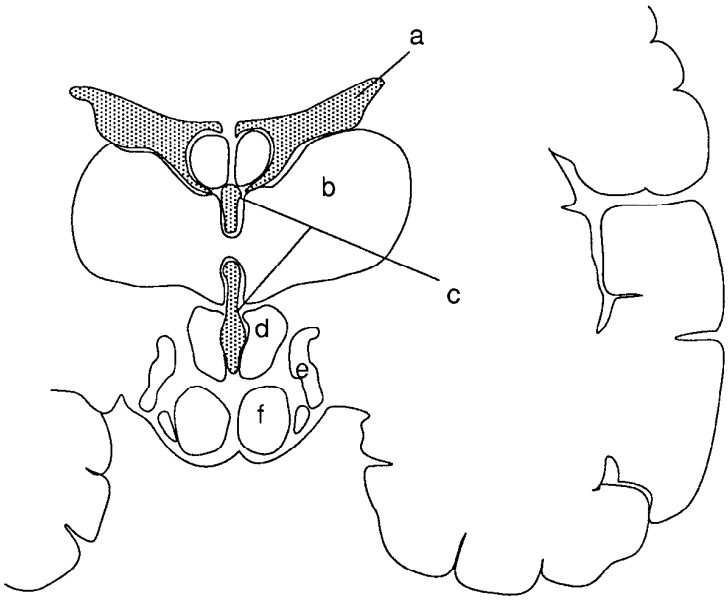

Fig. 6. The hypothalamic and adjacent structures of the posterior or mammillary groupings. *a*, lateral ventricle; *b*, thalamus; *c*, third ventricle; *d*, posterior hypothalamic area; *e*, lateral hypothalamic area; *f*, mammillary body.

the circadian changes in the timing of certain hormone systems such as those pituitary hormones that control the adrenal cortex (ACTH) and the reproductive system (LH and prolactin). The *periventricular nuclei* (Fig. 5A, part *e*) have small perikarya, which contain some of the release and release-inhibiting factors controlling the pituitary gland. Also associated with the anterior hypothalamic area are the morphologically indistinct telencephalic structures known as *circumventricular organs* (CVOs). One of these is the *organum vasculosum of the lamina terminalis (OVLT)*, and the other is the *subfornical organ (SFO)*. These are areas where the blood–brain barrier is absent, and thus can sense plasma osmolality. These areas play a role in the regulation of blood pressure and thirst because they contain angiotensin II (AII) receptors, and may even be able to synthesize their own AII. The OVLT has also been implicated in the control of LH secretion by GnRH. The other "*leaky*" CVOs are the *subcommissural organ* (SCO) and the *area postrema* (AP).

In the medial area the optic tracts separate, the lateral hypothalamus continues caudally, and the caudal termination of the supraoptic nucleus is located. Also in this area, the anterior hypothalamic area ends (Fig. 5A), and is replaced by two distinct nuclei, the *dorsomedial* and *ventromedial nuclei* (VMN) (Fig. 5B, parts *e*,*g*). The two can be separated by the small cells of the dorsomedial nucleus and the dense grouping of the neurons in the VMN. Both of these nuclei play a role in food intake. Lesions of both nuclei cause hyperphagia. Thus, this area regulates food intake in an inhibitory manner. The VMN in particular contains glucose-sensitive cells, which are believed to be the site through which caloric intake is monitored. The cells of the VMN are also rich in receptors for the gonadal steroids estrogen and testosterone, and thus are believed to play a major role in reproductive behavior and regulation of hormone secretion from the adenohypophysis. Finally, the *arcuate nucleus* begins in this medial hypothalamic area (Fig. 5A, part *l*; Fig. 5B, part *j*). The parvicellular neurons in this area have short axons, and some of these terminate on the *primary capillary bed* of the hypothalamo-hypophyseal portal system. The primary capillary bed is located in the underlying *median eminence* (ME) (Fig. 5A, part *m*). The neurons of the arcuate nucleus have several functions. In some mammals—such as the guinea pig, human, most monkeys bats, ferrets, cows, and the horse, cat, dog, and rabbit—GnRH neurons are in the basal portion of the medial hypothalamus. However, in others such as the rat and sheep, this area is devoid of GnRH neurons, or small numbers of such neurons are found in a so-called *cell-poor zone*. In those species in which the arcuate nucleus contains GnRH neurons, the fibers continue to the ME and some continue

through the infundibular stalk and into the neurohypophysis (human). A second function is in the control of prolactin and growth-hormone secretion. This area is populated by cells that contain dopamine, the prolactin-inhibiting hormone and growth hormone-releasing hormone (GHRH), the peptidergic stimulator of growth-hormone secretion. Finally, the arcuate nucleus is abundant in cells that contain β-endorphin, the endogenous opioid that is a cleavage product of the larger peptide, pro-opiomelanocortin (POMC). These neurons project to various hypothalamic and forebrain sites, and are believed to play a role in emotional behavior as well as endocrine function.

The posterior hypothalamic area contains the continuation of the lateral hypothalamic area as well as the *posterior hypothalamic nuclei and mammillary bodies* (Fig. 6, parts *d*,*f*). The posterior hypothalamic nucleus contains both small and large cell bodies, which give rise to efferent fibers descending through the central gray matter as well as the reticular formation (RF) of the brainstem. These neurons are believed to play a role in temperature regulation because they respond to cooling with the induction of shivering as well as the burning of brown adipose tissue. The mammillary nucleus is actually a complex consisting of medial and lateral nuclei. The mammillary bodies are critical circuits that link the hypothalamus with the limbic forebrain and midbrain structures lying rostral and caudal, thus implying a role in hypothalamic activity.

2.3. The Afferent and Efferent Connections are the Information Pathways of the Hypothalamus

The afferent and efferent connections of the hypothalamus reveal that this part of the brain is a complex integration center for somatic, autonomic, and endocrine functions.

2.3.1. Intrinsic Tracts

There are two main intrinsic tracts in the hypothalamus (*see* Fig. 1). The *infundibular tract* arises from neurons in the arcuate nucleus and periventricular nucleus with terminals on capillaries within the ME. These tracts axonally transport substances such as dopamine to the portal vessels. The *hypothalamo–hypophyseal tract* arises in the supraoptic and paraventricular nuclei and terminates in the neurohypophysis. As noted earlier, these axons transport vasopressin and OT, respectively. Both of these transfer information unidirectionally, from the hypothalamus to the pituitary gland.

2.3.2. Extrinsic Tracts

The lateral hypothalamus is reciprocally connected with the *thalamus*, the *paramedian mesencephalic area (limbic midbrain area)*, and the *limbic system*. The medial hypothalamus also receives connections from the limbic system (Fig. 7). It is quite clear that higher cortical centers communicate with the hypothalamus through the limbic system. In addition to the hypothalamus, the limbic system includes the *hippocampus*, the *amygdala*, the *septal area*, the *nucleus acumbens* (part of the *striatum*), and the *orbitofrontal cortex*. Anatomically, the hypothalamus is intimately related to the amygdala, which sits in the temporal lobe just rostral to the hippocampus. Efferents from the amygdala enter the hypothalamus through the *ventral amygdalofugal pathway*. The rostral amygdalofugal fibers form the *diagonal band of Broca*. More caudally, these fibers fan out and enter the hypothalamus, and many of them terminate near the VMN. A second afferent to the hypothalamus arises from the *corticomedial amygdala*. This pathway, the *stria terminalis*, terminate near the VMN of the hypothalamus. The other major limbic afferent to the hypothalamus arises from the hippocampus. The body of the hippocampus gives rise to the columns of the *fornix*, which courses toward the *anterior commissure* and then splits into two portions. The *post-commissural fornix* terminates in the mammillary bodies at the caudal end of the hypothalamus. Arising from the mammillary bodies is the *mammillothalamic tract*, which extends to the anterior nuclei of the thalamus, and then projects to the cingulate gyrus and the parahippocampal gyrus before returning to the hippocampus. A second efferent projection from the mammillary bodies, the *mammillotegmental tract*, turns caudally to the ventral tegmentum. A reciprocal pathway from the ventral tegmentum to the mammillary bodies is the *mammillary-pudendal tract*. The *Dorsal-longitudinal fasciculus of Schütz* are efferents from the periventricular nuclei of the hypothalamus, which terminate in the mesencephalic central gray. Stimulation of this fiber bundle produces fear and adverse reactions. A subset of ganglion cells in the retina projects to the suprachiasmatic nucleus of the hypothalamus by way of the *retinohypothalamic tract*. This tract transmits lighting periodicity information to be transduced by the

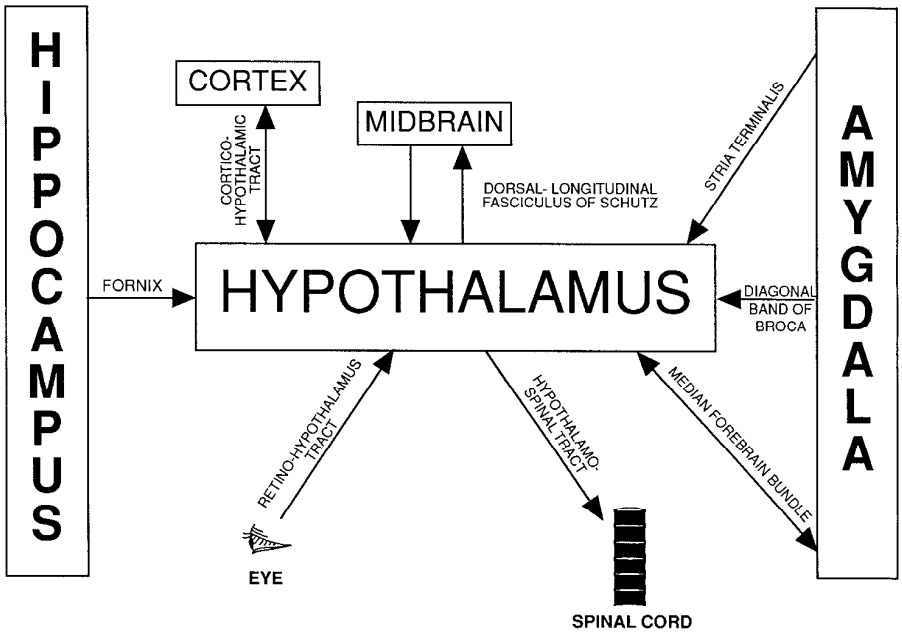

Fig. 7. A diagrammatic representation of some afferents and efferents of the hypothalamus.

suprachiasmatic nucleus. The *hypothalamospinal tract* originates in the supraoptic and paraventricular nuclei (parvicellular division), which projects down the spinal cord to the thoracic level and terminates in the intermediolateral column, and from there to the preganglionic sympathetic nerves. Based upon the anatomy, this pathway clearly must be important as the pathway over which the hypothalamus influences autonomic function. The other major hypothalamic fiber tract is the *median forebrain bundle*. This is a collection of tracts with ascending and descending fibers that run in the lateral hypothalamus between the midbrain RF and the basal forebrain. The descending fibers originate from structures in the basal forebrain, including the olfactory cortex, the preoptic area, the septal area, the accumbens, and the amygdala. The ascending portion comes from spinal cord and RF and visceral and taste nuclei in the brainstem as well as monoaminergic centers in the brainstem.

2.4. Blood Flow as a Means of Communicating Hypothalamic Information

The key neurohumoral link between the hypothalamus and the pituitary gland is the *hypothalamo–hypophyseal portal vasculature* (Fig. 8), which arises from a *primary capillary plexus* that extends from the ME to the adenohypophysis. This plexus is supplied with blood from three sources: rostrally by the *superior hypophyseal artery*, caudally by the *inferior hypophyseal artery* and mediorostrally by the *anterior hypophyseal artery (or Trabecular artery)*. All three of these arise from the *internal carotid artery*. In some species (rat, rabbit, and cat) they unite to form a single artery that supplies the infundibular stem. These arteries encircle the ME. The inferior hypophyseal artery also supplies the neurohypophysis. The primary capillary plexus in the ME is drained by the fenestrated *long portal vessels*, which course to the adenohypophysis, where they branch to a *secondary capillary plexus*. The primary capillary plexus is the site at which axon terminals converge to release their quanta of hypophysiotropic peptides into portal blood. After transport through the long portal vessels, they are released from the secondary capillary plexus to the surrounding adenohypophyseal cells. A set of *short portal vessels* arise from the anterior hypophyseal artery. These connect the infundibular stem, the neurohypophysis, and the intermediate lobe of the pituitary gland to the adenohypophysis. The short portal vessels are the route through which neurohypophyseal and intermediate-lobe peptides travel to the anterior pituitary.

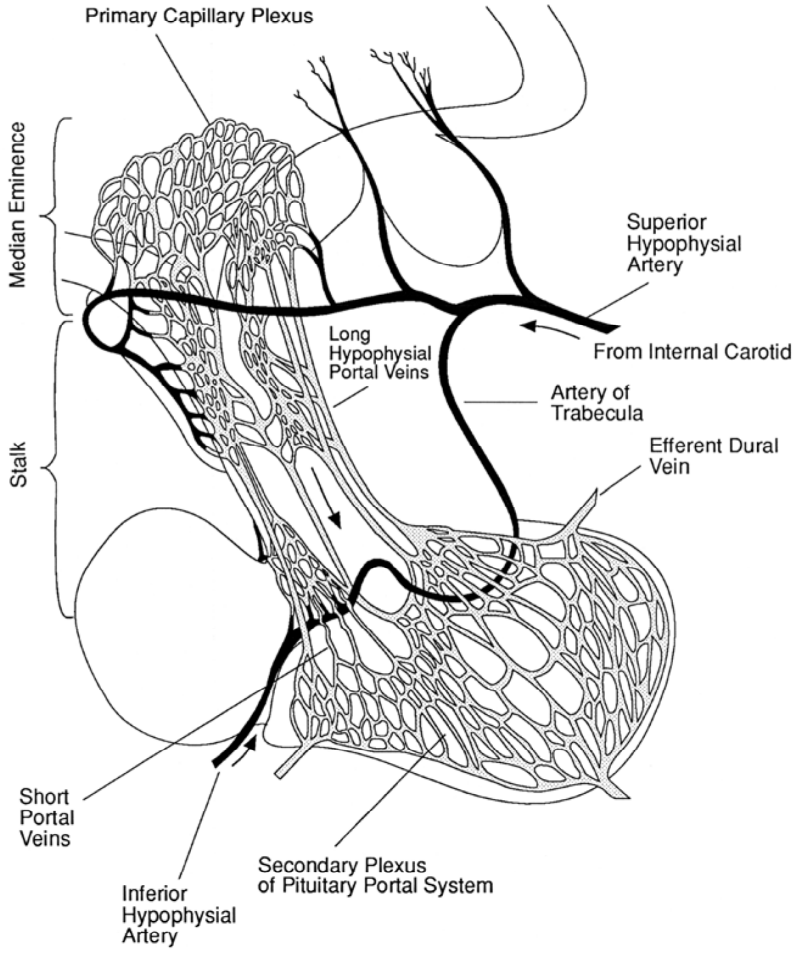

Fig. 8. A schematic representation of the hypothalamo–hypophyseal portal vasculature in man. *See* text for details.

2.5. The Chemiarchitecture Describes the Functions of the Hypothalamus

2.5.1. Monoamines

There are essentially three monoamines of importance to hypothalamic function, and their distributions have been described. These are dopamine, norepinephrine, and serotonin.

2.5.1.1. Dopamine. There are two major dopaminergic systems with long axons that originate from outside the hypothalamus (Fig. 9). The *nigrostriatal system*, has cell bodies in the substantia nigra with long axons that terminate in the caudate-putamen and globus pallidus. The *mesolimbic system*, has cell bodies in the ventral tegmentum that send projections through the hypothalamus and terminals in areas of the limbic system such as the nucleus acumbens, olfactory tubercle, cingulate cortex, and frontal cortex. Axons of both of these areas travel through the medial forebrain bundle. The hypothalamus contains three intrinsic dopaminergic pathways with short axons. The cell bodies of the *incertohypothalamic neurons* are located in the caudal hypothalamus, zona incerta, and rostral periventricular nucleus, with axons terminating in the dorsal hypothalamus, preoptic area, and septum. The cell bodies of the *tuberoinfundibular neurons* are located in the arcuate and periventricular nuclei, with short axons that terminate in the ME. These converge upon the primary capillary bed of the hypophyseal portal system, and thus have been shown to play a direct role in the release of hormones from the adenohypophysis. The *tuberohypophyseal neurons* have cell bodies in the rostral arcuate and periventricular nuclei, with axons terminating in the intermediate and posterior lobes of the pituitary gland. In the neurohypophysis, these axons lie in close proximity to vascular

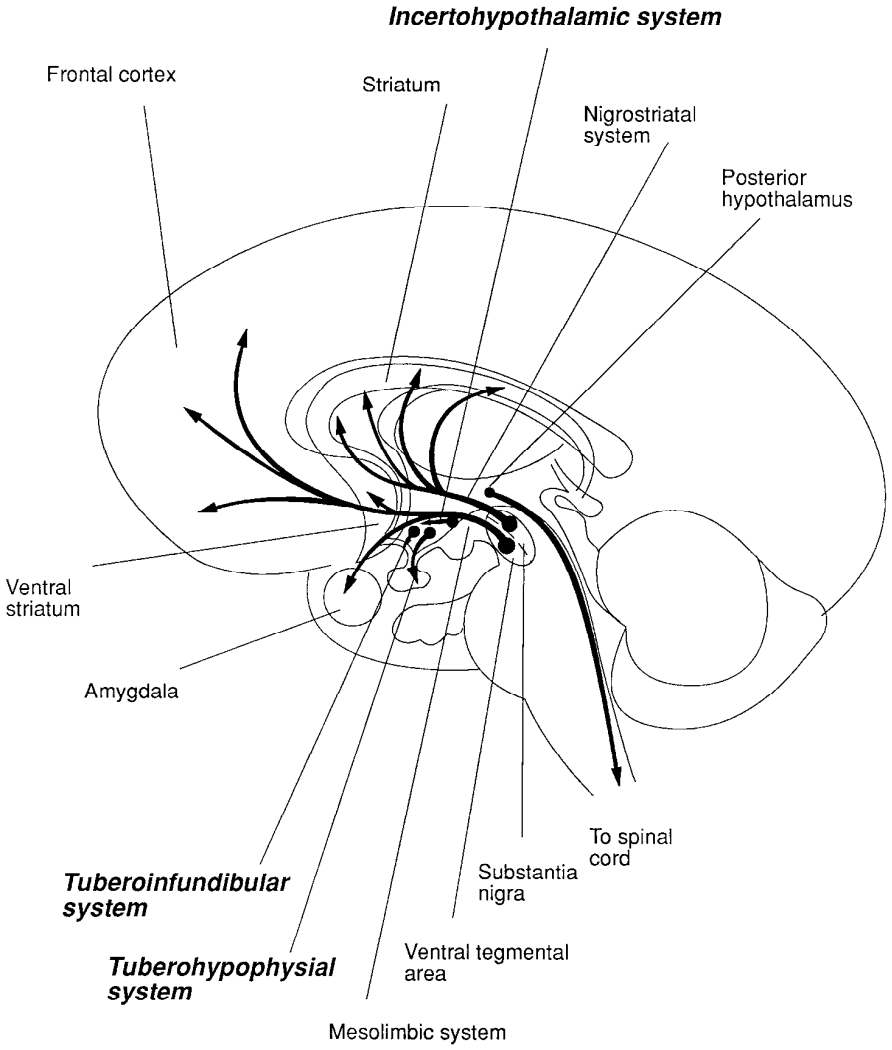

Fig. 9. Diagrammatic representation of the dopaminergic system. Two dopaminergic systems originate and terminate outside of the hypothalamus: the *nigrostriatal* and *mesolimbic*. The hypothalamus contains three intrinsic dopaminergic systems: (i) the *incertohypothalamic*, with cell bodies in the caudal hypothalamus, zona incerta and rostral periventricular nucleus with terminals in the rostral preoptic area and septum; (ii) the *tuberoinfundibular*, with cell bodies in the arcuate and periventricular nuclei and terminals in the external zone of the ME adjacent to the primary capillary bed; (iii) the *tuberohypophysial*, with cell bodies in the rostral arcuate and periventricular nuclei and terminals in the intermediate and posterior lobe of the pituitary gland. The tuberoinfundibular and tuberohypophysial dopaminergic systems are responsible for delivering dopamine to the adenohypophysis through the portal vasculature.

spaces, neurosecretory axons, and pituicytes (modified astroglial cells). Within the intermediate lobe, these axons terminate on secretory cells known as melanotropes. It is believed that a portion of the dopamine that acts within the adenohypophysis originates from the axon terminals in the intermediate and posterior lobes, and ultimately reaches the anterior pituitary through short portal vessels. Within the hypothalamus, the incertohypothalamic dopaminergic neurons appear to play a neuromodulatory role, and the tuberoinfundibular and tuberohypophyseal neurons subserve a neuroendocrine role.

2.5.1.2. Norepinephrine. The noradrenergic cell bodies of greatest importance to the hypothalamus are the *locus coeruleus* (Fig. 10). The efferents course toward the hypothalamus as the large *dorsal noradrenergic* (or *tegmental*) *bundle* and the *rostral limb of the dorsal periventricular pathway*. The former path-

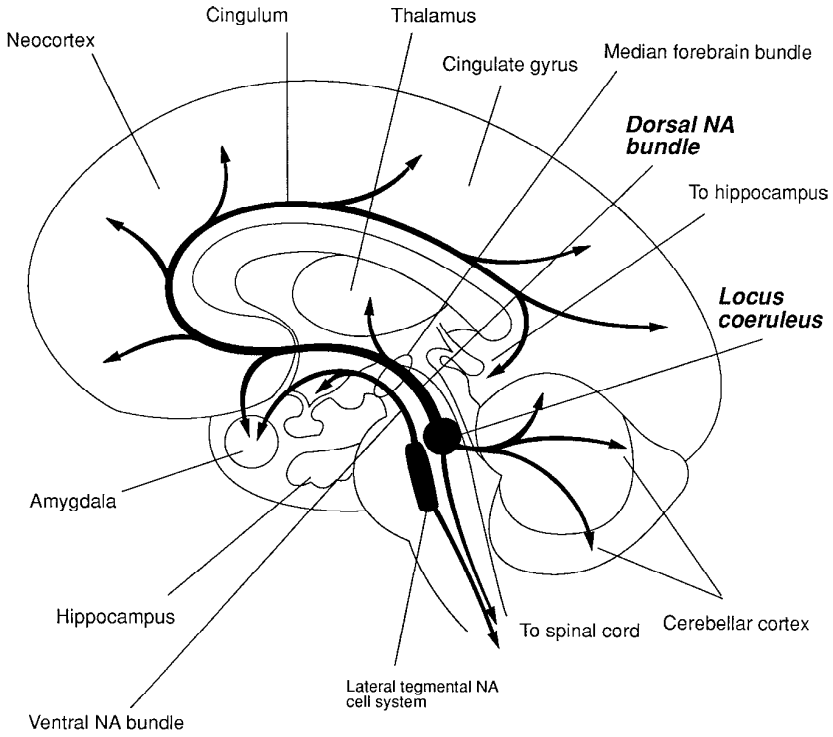

Fig. 10. Diagrammatic representation of the noradreneregic system. The cell bodies of greatest importance to the hypothalamus are located in the locus coeruleus, whose efferents course toward the hypothalamus as the dorsal noradrenergic bundle. These terminate in the paraventricular and arcuate nuclei of the hypothalamus as well as in the preoptic area.

way joins the ascending *ventral noradrenergic bundle* from the *lateral tegmental noradrenergic* cell groups. The dorsal and ventral noradrenergic pathways unite in the *median forebrain bundle* to enter the amygdala (dorsal) and hypothalamus (ventral).

2.5.1.3. Serotonin. Two groups of serotonergic cell bodies are found in the brain, in the *dorsal* and *medial raphe nuclei* (Fig. 11). Axons from the dorsal raphe nucleus form the *ventral ascending serotonergic pathway*, which sweep ventrally and then curve rostrally through the ventral tegmentum to join noradrenergic fibers of the median forebrain bundle in the lateral hypothalamic area. Two large fiber groups leave the ventral ascending pathway as it courses through the lateral hypothalamus—one directed laterally and the other ventromedially. The ventromedial fibers innervate many hypothalamic areas, including the lateral, medial preoptic, and anterior hypothalamic areas as well as the dorsomedial and VMN, the infundibulum, and the suprachiasmatic nuclei. In addition, the OVLT is rich in serotonin terminals.

2.5.2. Peptides

Time-honored steps must be taken to identify peptides as physiologically significant in the hypothalamus (Table 3). First, a quantitative bioassay must be established. A specific, dose-dependent relationship must be established between the amount of peptide and the biological response. Second, evidence must be provided that the biologically active material is peptidic in nature. This can be established by demonstrating that proteolytic enzymes diminish or destroy the biological activity. Third, a scheme for extraction and separation of maximal yields of the purified peptide must be devised. Fourth, chemical and physical characterization of the peptide must be performed. This would consist of mol-wt characterization as well as amino acid composition and sequencing. Fifth, once the sequence is known, the peptide must be synthesized and the synthetic product must be tested for biological activity in the bioassay. Sixth, antibodies to the peptide must be produced, and the purified antibodies must be characterized using synthetic analogs of the

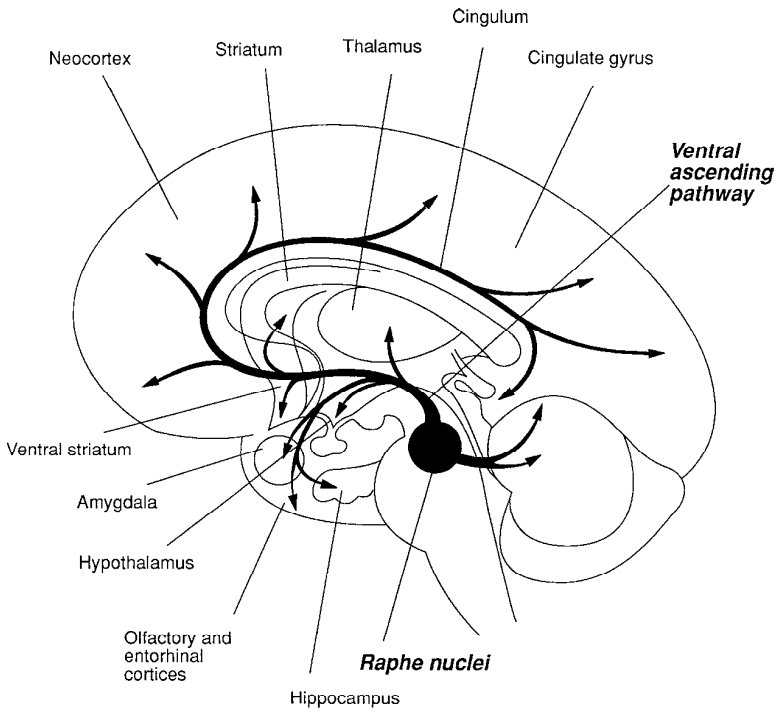

Fig. 11. Diagrammatic representation of the serotonergic system. The cell bodies of importance to hypothalamic function are found in the dorsal and medial raphe nuclei. Axons from the dorsal raphe form the ventral ascending serotonergic pathway, and enter the hypothalamus ventromedially to terminate in the anterior hypothalamus, dorsomedial and ventromedial nuclei, the suprachiasmatic nuclei, and the infundibulum. In addition, serotonergic terminals are found in the lateral and medial preoptic areas as well as the OVLT.

Table 3
Strategies in the Analysis of a Hypothalamic Neuropeptide

1.	Development of quantitative bioassay.
2.	Proof of peptidic nature.
3.	Development of extraction scheme.
4.	Chemical and physical characterization.
5.	Synthesize peptide and determine its bioactivity.
6.	Produce antibodies to the peptide.
7.	Use antibodies for immunocytochemical localization and radioimmunoassay.
8.	Isolate the cDNA encoding the precursor of the peptide.

peptide. Several immunologic approaches with the antibodies must be developed. This would consist of immunocytochemistry for visualization of peptides in neural tissue, as well as radioimmunoassay for quantitation of the concentration of the peptide in neural tissue and portal blood. Finally, the cDNA that encodes the precursor of the peptide must be isolated, and methods such as *in situ* hybridization histochemistry and Northern blotting must be developed for detecting the mRNA of the precursor. Most of these approaches have been taken to identify the peptides of importance to hypothalamic function.

Six arbitrary classes of peptides are involved in hypothalamic function (Table 4): *hypophysiotropic peptides* (Fig. 12), which affect the function of the adenohypophysis; the *neurohypophyseal peptides* (Fig. 13), which control blood pressure, water retention, milk ejection, and smooth-muscle contraction;

Table 4
Classes of Hypothalamic Peptides

Class	*Function*	*Example*
1. Hypophysiotropic peptides	Regulate adenohypophysis.	TRH, GnRH, GHRH, CRH, somatostatin
2. Neurohypophysial peptides	Regulate water retention, blood pressure, milk ejection, uterine contraction.	Vasopressin, oxytocin
3. Brain-gut peptides	Neuromodulatory, neuroendocrine.	VIP, CCK, substance P
4. POMC-derived peptides	Neuromodulatory, neuroendocrine.	Endorphins, ACTH
5. Dynorphin-derived peptides	Neuromodulatory, neuroendocrine.	Met-enkephalin, leu-enkephalin
6. "Other" peptides	Neuromodulatory, neuroendocrine.	Angiotensin II, NPY, galanin, endothelins

LHRH: p[1]Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly[10]-NH_2

TRH: p[1]Glu-His-Pro[3]-NH_2

CRH: [1]Ser-Gln-Glu-Pro-Pro-Ile-Ser-Leu-Asp-Leu-Thr-Phe-His-Leu-Leu-Arg-Glu-Val-Leu-Glu-Met-
(ovine) Thr-Lys-Ala-Asp-Gln-Leu-Ala-Gln-Gln-Ala-His-Ser-Asn-Arg-Lys-Leu-Leu-Asp-Ile-Ala[41]NH_2

GHRH: [1]Tyr-Ala-Asp-Ala-Ile-Phe-Thr-Asn-Ser-Tyr-Arg-Lys-Val-Leu-Gly-Gln-Leu-Ser-Ala-Arg-Lys-Leu-Leu-Gln-Asp-Ile-Met-Ser-Arg-Gln-Gln-Gly-Glu-Ser-Asn-Gln-Glu-Arg-Gly-Ala-Arg-Ala-Arg-Leu[44]-NH_2

Somatostatin: [1]Ala-Gly-Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys[14]

Fig. 12. Amino acid sequences of the *hypothalamic hypophysiotropic peptides*, so named because they all regulate pituitary function directly. The superscript "1" represents the amino terminus, and the carboxyl terminus is designated with the greater suprascript number. Note that the Glu at position 1 of LHRH and TRH is designated pyro (p) and that LHRH, TRH, CRH, and GHRH all are amidated at their carboxy termini.

Arginine Vasopressin: [1]Cys-Tyr-Phe-Glu-Asn-Cys-Pro-Arg-Gly[9]-NH_2

Oxytocin: [1]Cys-Tyr-Ile-Glu-Asn-Cys-Pro-Leu-Gly[9]-NH_2

Fig. 13. Amino acid sequences of the *neurohypophyseal peptide* hormones. Note that they share common features: both are nonapeptides, have an intramolecular disulphide bond and are amidated at the carboxyl terminal. The differences that account for their differing bioactivities are found at positions 3 and 8. In addition, a closely related *pressor peptide*, lysine vasopressin, differs from AVP by substituting lys for arg in position 8.

brain-gut peptides (Fig. 14), which serve a predominantly neuromodulatory function; the *POMC-derived peptides* (Fig.15), which also subserve a neuromodulatory role in hypothalamic function; the *enkephalins* (Fig. 16), which also serve as modulatory peptides that are found in the hypothalamus and those found outside of the hypothalamus that effect hypothalamic function. Other peptides, such as *angiotensin II*, neu-

Substance P: ^{1}Arg-Pro-Lys-Pro-Glu-Glu-Phe-Phe-Gly-Leu-Met^{11}-NH_2

VIP: ^{1}His-Ser-Asp-Ala-Val-Phe-Thr-Asp-Asn-Tyr-Thr-Arg-Leu-Arg-Lys-Glu-Met-Ala-Val-Lys-Lys-Tyr-Leu-Asn-Ser-Ile-Leu-Asn^{2}-NH_2

CCK (8): ^{1}Asp-Tyr-Met-Gly-Trp-Met-Asp-Phe^{8}-NH_2
SO_3

NT: p^{1}Glu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu^{13}

Fig. 14. Amino acid sequences of the hypothalamic *brain-gut peptides*, so named because they are localized and active in both the brain and gastrointestinal system.

ACTH: ^{1}Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-Gly-Lys-Lys-Arg-Arg-Pro-Val-Lys-Val-Tyr-Pro-Asn-Gly-Ala-Glu-Asp-Glu-Leu-Ala-Glu-Ala-Phe-Pro-Leu-Glu-Phe^{39}

β -LPH: ^{1}Glu-Leu-Thr-Gly-Gln-Arg-Leu-Arg-Glu-Gly-Asp-Gly-Pro-Asp-Gly-Pro-Ala-Asp-Asp-Gly-Ala-Gly-Ala-Gln-Ala-Asp-Leu-Glu-His-Ser-Leu-Leu-Val-Ala-Ala-Glu-Lys-Lys-Asp-Glu-Gly-Pro-Tyr-Arg-Met-Glu-His-Phe-Arg-Trp-Gly-Ser-Pro-Pro-Lys-Asp-Lys-Arg-Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu-Phe-Lys-Asn-Ala-Ile-Ile-Lys-Asn-Ala-Tyr-Lys-Lys-Gly-Glu^{89}

α-MSH: Ac-^{1}Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val^{13}-NH_2

γ-LPH: ^{1}Glu-Leu-Thr-Gly-Gln-Arg-Leu-Arg-Glu-Gly-Asp-Gly-Pro-Asp-Gly-Pro-Ala-Asp-Asp-Gly-Ala-Gly-Ala-Gln-Ala-Asp-Leu-Glu-His-Ser-Leu-Leu-Val-Ala-Ala-Glu-Lys-Lys-Asp-Glu-Gly-Pro-Tyr-Arg-Met-Glu-His-Phe-Arg-Trp-Gly-Ser-Pro-Pro-Lys-Asp^{56}

β -END: ^{1}Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu-Phe-Lys-Asn-Ala-Ile-Ile-Lys-Asn-Ala-Tyr-Lys-Lys-Gly^{31}

β -MSH: ^{1}Asp-Glu-Gly-Pro-Tyr-Arg-Met-Glu-His-Phe-Arg-Trp-Gly-Ser-Pro-Pro-Lys-Asp^{18}

γ-MSH: ^{1}Tyr-Val-Met-Gly-His-Phe-Arg-Trp-Asp-Arg-Phe-Gly^{12}

Fig. 15. Amino acid sequences of the hypothalamic peptides derived from *proopiomelanocortin*.

Met-Enk: ^{1}Tyr-Gly-Gly-Phe-Met^{5}

Leu-Enk: ^{1}Tyr-Gly-Gly-Phe-Leu^{5}

Fig. 16. Amino acid sequences of the *enkephalins*. Note that the sole difference between them is in position 5.

ropeptide Y and the endothelins (Fig. 17) could stand as a class by themselves because they may play a variety of neuroendocrine, neuromodulatory, neurotransmitter, and hormonal roles.

2.5.2.1. Hypophysiotropic Peptides. Of all the neuropeptides that control the function of the adenohypophysis, the chemiarchitecture of *luteinizing hormone-releasing hormone (GnRH)* is perhaps the most

Table 5
Localization of the Hypophysiotropic Peptides

Peptide	*Cell Bodies*	*Fibers*	*Terminals*	*Present in portal blood*
GnRH	Medial septal nucleus, nucleus of the diagonal band of broca, bed nucleus region of stria terminalis, OVLT, medial preoptic nucleus, anterior hypothalamic area, arcuate nucleus, ME, olfactory tubercle.	Continuum from septal region to premammillary nucleus.	ME, neurohypophysis, suprachiasmatic nucleus, ependymal lining of ventricles, olfactory bulb, amygdala.	+
TRH	Periventricular area, paraventricular nucleus, dorsomedial/ventromedial nuclei, arcuate nucleus.	Paraventricular nucleus, periventricular hypothalamic area, dorsomedial nucleus perifornical region, nucleus accumbens, bed nucleus of stria terminalis, spinal cord.	ME	+
CRH	Paraventricular nucleus, supraoptic nuclei, arcuate nuclei dorsal raphe nucleus, hippocampus, OVLT, medial preoptic nucleus, bed nucleus of stria terminalis, locus coeruleus.	Septal nuclei, stria terminalis, median forebrain bundle.	ME	+
GHRH	Arcuate nucleus.		ME	+
SS	Preoptic/anterior hypothalamic area, paraventricular nucleus.		ME, suprachiasmatic nucleus.	+

+ = Yes, in concentrations greater than peripheral blood.
– = No, concentrations the same as or lower than peripheral blood.

widely studied. GnRH-positive cell-body fibers and terminals are not restricted to the hypothalamus. They are scattered over a continuum extending from the septal region from the septal region to the premammillary region (Table 5). These are found in the medial septal nucleus, the nucleus of the diagonal band of Broca, the bed nucleus of the stria terminalis, the OVLT, the medial preoptic nucleus, the anterior hypothalamic area, the supraoptic nucleus, and the arcuate nucleus. Cell bodies in the arcuate nucleus and the adjacent medial ME area give rise to short axons, which pass to the infundibulum and form a dense plexus around the primary capillary bed of the hypothalamo-hypophyseal portal system in the ME. These are the cells that control the secretion of LH from the adenohypophysis. In some mammals, these same cells have axons that terminate directly in the neurohypophysis. These axons are of unknown function, but their termini in the neurohypophysis adjacent to the adenohypophysis suggests that they may play a role in adenohypophyseal function. GnRH-positive cells in the medial preoptic nucleus send projections to the suprachiasmatic nucleus and the ME. Cells in the medial septal nucleus and the diagonal band of Broca also contribute to this projection. Other GnRH-positive cells from these areas terminate in the OVLT to form a dense plexus around the capillary network, suggesting another vascular route through which the peptide reaches the anterior pituitary. GnRH-positive cells originating in the medial preoptic nucleus, the bed nucleus of the stria terminalis, and the septum send fibers that terminate on the ependymal linings of the third and lateral ventricles. This suggests that the cerebrospinal fluid (CSF) may be an additional vehicle for transport of GnRH. In the medial septal nucleus, the diagonal band of Broca, and the olfactory tubercle, a few groups of GnRH-positive cells terminate upon blood vessels in this area. In addition, GnRH-positive cells in the medial preoptic nucleus, medial septal nucleus, and the diagonal band of

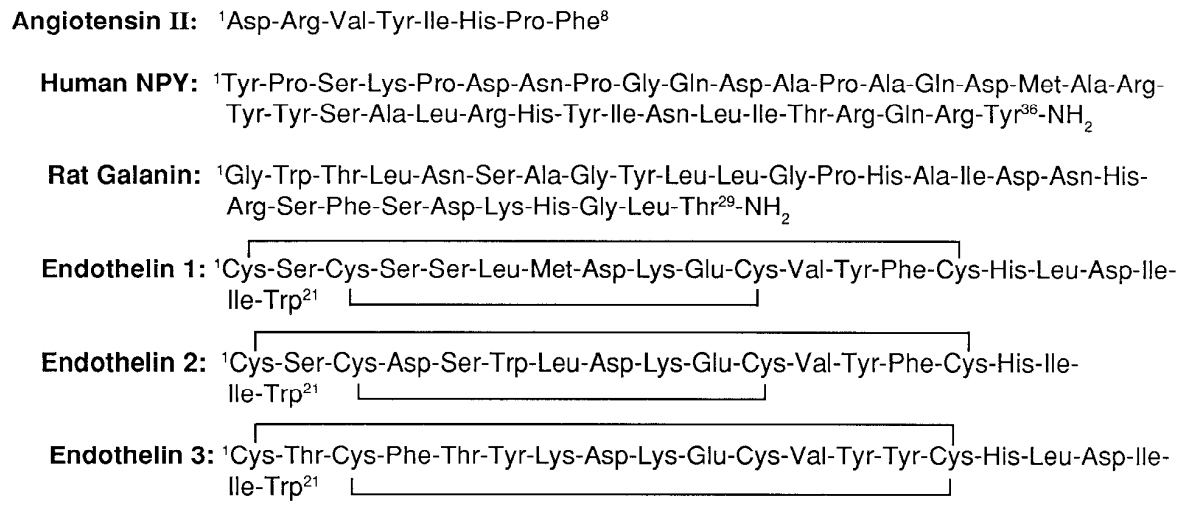

Fig. 17. Amino acid sequences of some of the *other peptides* localized in the hypothalamus and controlling hypothalamic function. Among the unusual features, note that the endothelins have two intramolecular disulphide bonds.

Broca terminate in the external plexiform and glomerular layers of the olfactory bulb. The medial preoptic nucleus also provides fibers to the amygdala through the stria terminalis. Although the GnRH neurons originating in the preoptic and arcuate nuclei that terminate in the ME have been shown to play a direct role in cyclic LH release, termini in other areas such as the olfactory system, the amygdala, the habenula, and the mesencephalic gray have not been assigned a firmly established functional role in reproductive processes. It is likely that these areas play a role in control of pituitary hormone secretion and the behaviors related to reproduction that are controlled by GnRH not as a neurohormone but as a neuromodulator. Finally, it is interesting to note that GnRH is not confined to the central nervous system (CNS). In the sympathetic ganglia of the bullfrog, a peptide that resembles GnRH and is co-localized with acetylcholine elicits prolonged excitatory postsynaptic potentials (EPSPs) with long latencies. This would categorize GnRH as a neurotransmitter.

The *Thyrotropin-Releasing Hormone (TRH)*, the physiological stimulator of TSH release from the adenohypophysis, is found widely throughout the nervous system of mammals (Table 5). In fact, only approximately one-third of the total amount of the peptide found in the brain is localized to the hypothalamus. Thus, TRH is believed to play both a neuromodulatory and neuroendocrine role. The highest concentration of TRH in the hypothalamus is found in the ME with significant levels in the dorsomedial, ventromedial, and arcuate nuclei. Extrahypothalamic structures such as the preoptic and septal areas as well as the motor nuclei of some cranial nerves also contain significant levels of TRH. Within the hypothalamus, TRH-positive cell bodies are found in the periventricular area, the paraventricular nucleus, the dorsomedial and ventromedial nuclei, and the arcuate nucleus/ME area. TRH-positive nerve terminals are found in the greatest abundance in the external layer of the ME, with dense fiber networks in the parvicellular part of the paraventricular nucleus, the periventricular hypothalamic area, the dorsomedial nucleus, the perifornical region, the nucleus acumbens, and the bed nucleus of the stria terminalis. TRH-positive fibers are also found in the spinal cord.

Corticotropin-releasing hormone (CRH), the physiological stimulator for the release of ACTH from the adenohypophysis, has been localized in hypothalamic and extrahypothalamic sites (Table 5). CRH-positive cells are found in greatest abundance in the paraventricular nucleus of the hypothalamus. Although most of the cells are parvicellular, some are members of the magnocellular population of this nucleus. Axons from the CRH-positive cells of the paraventricular nucleus project to a so-called *neurohemal area* that encompasses the external zone of the ME. These cells provide the CRH to the portal vasculature, bathing the adenohypophysis and stimulating the release of ACTH and β-endorphin. CRH-positive cell bodies have also been identified in the supraoptic and arcuate nuclei in the hypothalamus, with additional groups of cell bod-

ies in the dorsal raphe nucleus, the hippocampus, and the OVLT. Scattered CRH cell bodies are found throughout the lateral preoptic lateral hypothalamic continuum, and numerous cell bodies have been identified in the medial preoptic nucleus and the bed nucleus of the stria terminalis. In the midbrain, CRH-staining cells are found in the reticular formation (RF) and the periaqueductal gray. Moreover, CRH has been co-localized with the catecholamines in the locus coeruleus. CRH-immunoreactive fibers originating from the bed nucleus of the stria terminalis enter the lateral and medial septal nuclei. Numerous fibers found within the stria terminalis and the ventral amygdalofugal pathway connect the rostral hypothalamus and basal telencephalon with the amygdala. Fibers originating from the telencephalon/diencephalon course caudally through the median forebrain bundle and split into a dorsal pathway throughout the brainstem and a ventral pathway to the lateral part of the RF. Although a well founded physiological role has been ascribed to the hypothalamic paraventricular-ME pathway, a role for the other pathways has not been similarly characterized. Since ACTH-immunoreactive cell bodies and fibers of unknown function have been found throughout the brain localized closely with CRH, it is possible that this reflects the same regulatory relationship described for pituitary ACTH.

Growth hormone-releasing hormone (GHRH), the physiological stimulator of growth-hormone secretion from the adenohypophysis, has been found in hypothalamic, non-hypothalamic, and even non-neural sites (Table 5). The concentration of immunoreactive GHRH in the hypothalamus is highest in the arcuate nucleus/ME area, which is probably a reflection of its neuroendocrine role at the pituitary gland. Immunocytochemical localization studies have revealed GHRH-positive cell bodies in the arcuate nucleus, with short axons terminating in the arcuate nucleus and the ME. Some of these GHRH cell bodies also contain neurotensin, and others contain galanin. The physiological significance of this dual packaging is unknown. Interestingly, surgical isolation of the medial basal hypothalamus does not lead to a significant decline in the concentration of GHRH in the arcuate nucleus ME area. Thus, virtually all of the hypothalamic GHRH originates from cells in this area. Significant amounts of GHRH are also found in some nonneural sites. Specifically, both GHRH-messenger RNA and newly synthesized GHRH are found in somatotropes of the adenohypophysis. This has led to the belief that some degree of growth-hormone secretion is intrinsic through an autocrine relationship. Other locations of GHRH cells lack a compelling physiological explanation. GHRH has been found in the ovary and the placenta—sites for which a role for GHRH has yet to be described.

Growth hormone release-inhibiting hormone or somatostatin (SS), as its name implies, inhibits the secretion of growth hormone or somatotropin from the adenohypophysis. However, the name does not fully represent the variety of roles played by this neurohormone. SS also inhibits the release of thyrotropin and prolactin from the adenohypophysis. In addition to its location in the hypothalamus, SS is widely distributed throughout the CNS (Table 5), suggesting that it may be a neurotransmitter or neuromodulator as well as a neurohormone. Of interest to control of growth-hormone secretion, SS-positive cell bodies are quite abundant in the preoptic-anterior hypothalamic area. Parvicellular SS-positive cells are also found in the paraventricular nucleus. These areas send axons as a group caudally to terminate in the suprachiasmatic nucleus, as well as the arcuate nucleus/ME area. Fibers pass from the preoptic area terminate in the primary capillary bed of the ME. This is the source of SS, which directly inhibits growth-hormone secretion from the adenohypophysis. Moreover, these same preoptic SS fibers synapse on GHRH cell bodies in the arcuate nucleus. This suggests two levels of inhibition of growth-hormone secretion by SS: directly at the somatotrope and secondarily at the GHRH neuron. Finally, SS can be found outside of the nervous system. Within the endocrine pancreas a specific cell type—the delta cell—synthesizes and secretes SS that is identical to that made by hypothalamic neurons. Pancreatic SS plays numerous roles in the gastrointestinal tract. In addition, SS directly affects pancreatic insulin and glucagon secretion.

2.5.2.2. Neurohypophyseal Hormones. *Arginine-vasopressin (AVP)*—which is also known as *antidiuretic hormone (ADH)*—and another neurohormone, *oxytocin (OT)*, are produced in magnocellular (e.g., large) neurons whose cell bodies are located in the *supraoptic* (AVP) and *paraventricular* (OT) nuclei of the hypothalamus (Table 6). They are synthesized as *prohormones*, or precursor proteins, in the cell body (Fig. 18). These large molecules consist of packaging peptides and specific axonal transport peptides, *neurophysins (NP)*, as well as the bioactive fragment AVP or OT, which is ultimately found in the peripheral circulation. There are actually two forms of the NPs. NP-I, or estrogen-linked neurophysin, is

Table 6
Localization of Neurohypophyseal Peptides

Peptide	*Cell bodies*	*Fibers*	*Terminals*	*Presence in portal blood*
OT	Paraventricular nucleus, supraoptic nucleus.	Hypothalamo–hypophysial tract.	Neurohypophysis, ME.	+
AVP	Supraoptic nucleus, paraventricular nucleus, suprachiasmatic nucleus, bed nucleus of stria terminalis, nucleus of diagonal band of broca, amygdala.	Hypothalamo–hypophysial tract, septum, thalamus, hippocampus.	Neurohypophysis, ME OVLT, dorsomedial nucleus.	+

+ = Yes, in concentrations greater than peripheral blood.
– = No, concentrations the same as or lower than peripheral blood.

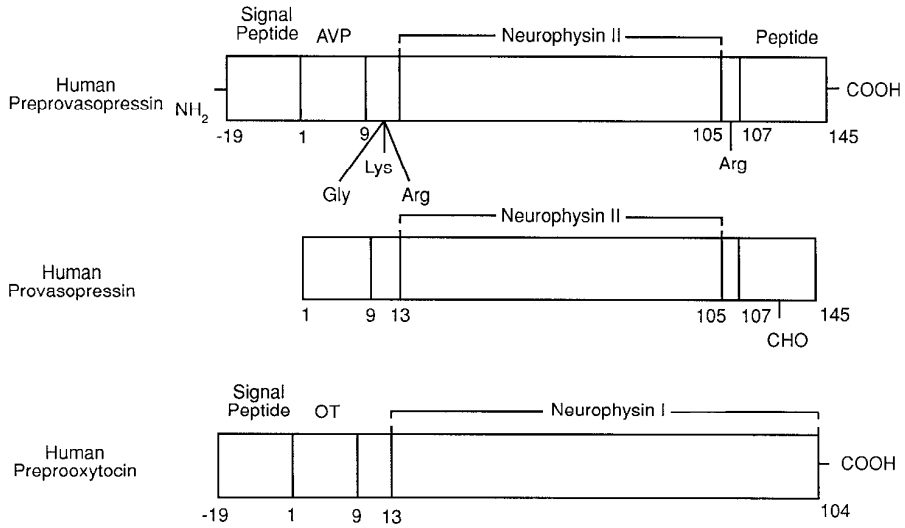

Fig. 18. The structure of human preprovasopressin, provasopressin, and preprooxytocin. Note that preprovasopressin is 21 K_d and consists of a signal peptide, the AVP sequence; a tripeptide spacer, neurophysin II (also called nicotine-linked neurophysin); a spacer glycosylation signal (Arg), and a 39-amino-acid carboxyl terminal. Provasopressin is a 19-K_d product of preprovasopressin from which the signal sequence has been deleted and carbohydrate (CHO) added to the C-terminal peptide posttranslationally. Preprooxytocin is smaller than preprovasopressin, with a different neurophysin, and the posttranslational modifications do not include glycosylation.

associated with OT and NP-II, or nicotine-linked neurophysin is associated with AVP. Both NPs are 9.5–10 K_d The translation product of the AVP gene is a 21-K_d protein known as *preprovasopressin* (Fig. 18), which in humans consists of a 19-aa signal peptide at the amino terminal, vasopressin (9 aa), a 3-aa spacer sequence, NP-II (93 aa), another spacer sequence (1 aa), and a 39-aa peptide at the carboxyl terminal. *Provasopressin* (19 K_d) is the peptide with the 19-aa signal sequence cleaved and carbohydrate added post-translationally to the C-terminal peptide. Oxytocin is synthesized in a similar fashion, with the exception that there is no glycopeptide at the carboxyl terminus and the prohormone is significantly smaller—15 K_d (Fig. 18). In both cases, the NP is required as a carrier protein to the axon terminal and presumably prolongs the half-life of the neuropeptide. The peptide-NP complex is exocytosed into fenestrated capillaries, where

the respective NP is cleaved from either OT or AVP. Originally it was believed that the cell bodies of the supraoptic nucleus exclusively contained AVP, and the cell bodies of the paraventricular nucleus contained exclusively OT. We now know that both types of nuclei are found in each area. Axons from each of these nuclei form the *hypothalamo–hypophyseal tract*, which terminates upon sinusoids in the neurohypophysis. OT and AVP can also be transported from the neurohypophysis to the adenohypophysis through the short portal vessels connecting these two areas. The paraventricular nucleus also sends fibers to the ME, where they terminate upon the primary capillaries from which the long portal vessels project to bathe the adenohypophysis. This is the pathway by which AVP reaches the corticotrope and stimulates ACTH secretion. It is well-known that AVP is an *accessory* ACTH-releasing factor of hypothalamic origin. Also, the caudal portion of the paraventricular nucleus contains *parvicellular* or small neurons, which contain mostly OT and some AVP. These neurons also project to the ME as well as other parts of the brain and spinal cord. Therefore, significant quantities of OT are found in portal blood. The parvicellular part of the paraventricular nucleus also sends fibers to the locus coeruleus, the parabranchial nuclei, the dorsal motor vagal nucleus, the nucleus of the solitary tract, the midbrain central gray and the dorsal horn of the spinal cord. Localization of AVP and OT fibers in the lower brainstem and autonomic centers may reflect their roles in such peripheral processes as the regulation of blood pressure and lactation. Other parvicellular neurons are found in the suprachiasmatic nucleus. These almost exclusively contain AVP and project to the OVLT, the dorsomedial hypothalamic nucleus, and the thalamus. Also outside of the supraoptic and paraventricular nuclei, AVP cells have been found in the bed nucleus of the stria terminalis to the nucleus of the diagonal band of Broca and lateral septum, the anterior amygdala, the lateral habenular nucleus , the mesencephalic central gray, and the locus coeruleus. The location of AVP fibers in the septum is compatible with a role of the peptide in thermoregulation, and the presence of fibers in the mediodorsal thalamic nucleus, the hippocampus, and the neocortex is compatible with a role for this peptide in learning and memory.

2.5.2.3. Brain-Gut Peptides. This group of peptides is so named because the activities have been characterized by immunocytochemistry within the gastrointestinal tract as well as the hypothalamus (Table 7). In addition to many of them being localized in the hypothalamus, a neuroendocrine role for some have been identified.

Substance P has been found in extracts of the brain and intestine. Its structure is known, and it has been implicated in pain perception, baroreception, and chemoreception. In the monkey, substance P cell bodies have been found in the most lateral portions of the arcuate nucleus. Fibers pass to the external zone of the ME and also to the neurohypophysis. In the rat, substance P-positive cells are found in the medial and lateral preoptic areas, the anterior hypothalamic area, and the dorsomedial and ventromedial nuclei. Substance P-containing afferent pathways project to the supraoptic and paraventricular nuclei as well as the arcuate nucleus. However, despite the location of substance P fibers in the neurohemal zone of the ME, the levels of substance P in portal blood is essentially equivalent to that of peripheral blood; thus eliminating it as a neurohormone. Substance P and its receptors are also found within the adenohypophysis. Alternatively, substance P may be found in the anterior lobe as a paracrine agent rather than as a neurohormone. Substance P plays a role in the secretion of all the important anterior pituitary hormones, either by acting directly on the specific cells of the gland or indirectly as a neuromodulator affecting the release of the releasing hormones of hypothalamic origin.

Vasoactive intestinal polypeptide (VIP) is present in large quantities throughout the gastrointestinal tract, where it plays multiple roles in digestive processes. For example, it is vasodilatory, glycogenolytic, and lipolytic. It enhances insulin secretion, inhibits gastric acid production, and stimulates secretion from the exocrine pancreas and small intestine. Since it exerts some of its effects through vascular pathways, it fits the description of a true hormone. Neuronal VIP is found in highest concentrations in the cerebral cortex, where it acts as an excitatory neurotransmitter or neuromodulator. Within the hypothalamus, the suprachiasmatic nuclei contain very dense concentrations of VIP-positive cell bodies. Efferents from these course dorsally and then split into a dense rostro-dorsal component and a less dense caudal component. The rostrodorsal fibers terminate on the paraventricular nucleus, and the caudal fibers terminate at the dorsomedial, ventromedial, and premammillary nuclei. Like substance P, VIP is found in high concentrations

Table 7
Localization of Brain-Gut Peptides

Peptide	*Cell bodies*	*Terminals*	*Presence in portal blood*
Substance P	Arcuate nucleus, preoptic area, anterior hypothalamic area, dorsomedial/ventromedial nuclei.	ME, neurohypophysis, supraoptic nucleus, paraventricular nucleus, arcuate nucleus.	+
VIP	Suprachiasmatic nucleus.	Paraventricular nucleus, dorsomedial/ventromedial nuclei.	+
CCK	Cortex, striatum, amygdala supraoptic nucleus, para-ventricular nucleus, neurohypophysis, preoptic area, dorsomedial nucleus.	ME.	–
NT	Preoptic area, anterior hypothalamic area, medial preoptic area, paraventricular nucleus, dorsomedial nucleus, arcuate nucleus.	ME, neurohypophysis.	+

+ = Yes, in concentrations greater than peripheral blood.
– = No, concentrations the same as or lower than peripheral blood.

in the adenohypophysis. However, unlike substance P, it is also found in high concentrations in portal blood. There is also evidence that it may be synthesized in the adenohypophysis. VIP has been shown to affect adenohypophyseal hormone secretion as a transmitter/neuromodulator, as a neurohormone, and as a local autocrine or paracrine agent.

Cholecystokinin (CCK) is synthesized in the duodenum, and stimulates the secretion of pancreatic enzymes and the ejection of bile from the gallbladder. Unfortunately, CCK occurs in multiple molecular forms, which complicates the description of its tissue distribution. Duodenal CCK is composed of 33 or 39 amino acids. The carboxy-terminal octapeptide of CCK (CCK8) which has full biological activity, shares a pentapeptide sequence with gastrin, another gastrointestinal hormone. However, for the most part, whereas CCK8 occurs throughout the CNS, gastrin-like peptides are found only in the pituitary gland and hypothalamus. The highest concentrations of immunoreactive CCK8 are found in the cortex, striatum, and amygdala, with lesser amounts in the hypothalamus. CCK-like immunoreactivity has been found in the magnocellular systems of the supraoptic and paraventricular nuclei of the hypothalamus, as well as the neurohypophysis. In some neurons, OT and CCK are co-localized. Physiological perturbations that stimulate the release of AVP and OT lower neurohypophyseal CCK. Parvicellular CCK-positive cells project axons that terminate in the ME. In addition to these, CCK cells are found in the medial preoptic area as well as the dorsomedial and supramammillary nuclei. Functionally, CCK has been implicated as a neuromodulator in the control of pituitary hormone release. As such, it probably facilitates the release of the hypothalamic-releasing hormones as well as OT and AVP. It has also been shown that CCK may be copackaged with many of the hypothalamic peptides. There is no definitive direct evidence implicating CCK as a neurohormone.

Neurotensin (NT) is a peptide consisting of 13 amino acids, which was first isolated from the brain and later from the intestine. In general, NT is a peptide neurotransmitter is found in highest concentrations in the hypothalamus, and seems to play a role in the control of release of the adenohypophyseal hormones.

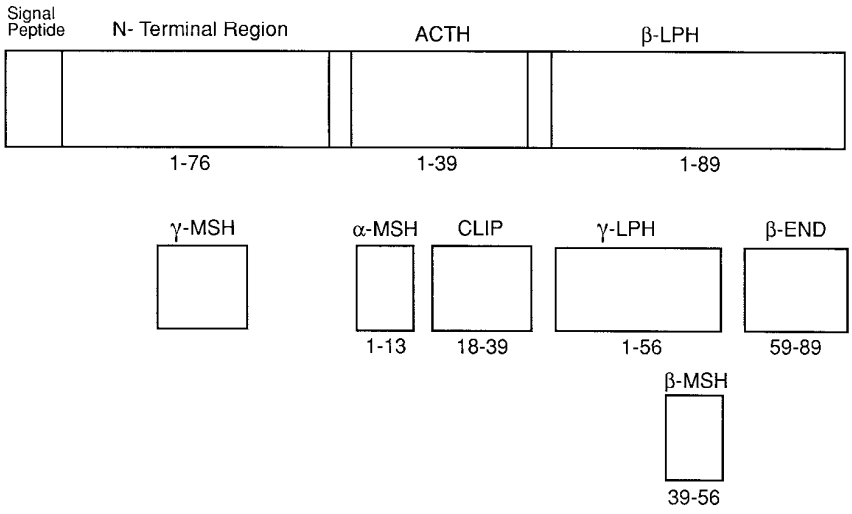

Fig. 19. Human POMC and its cleavage products. The numbers below each bar represent the number of amino acids of the parent peptides (*upper bar*) or the position in the parent peptides from which the products are cleaved (*middle* and *lower bars*).

NT-positive cell bodies are found in the preoptic and anterior hypothalamic areas, the medial preoptic nucleus, the magnocellular and parvicellular zones of the paraventricular nucleus, the arcuate nucleus, and the dorsomedial nucleus. In the arcuate nucleus, NT is colocalized in tuberoinfundibular dopaminergic neurons. The biological significance of this common packaging is not yet fully appreciated. However, it has been suggested that NT mediates the release of dopamine into the portal vasculature. NT-positive fibers are also localized to the external zone of the ME. In addition, NT is present in portal blood, and thus may assume the role of a classic neurohormone. When placed into the brain, NT can affect the secretion of prolactin, growth hormone, TSH, and LH, yet its placement directly into pituitary-cell cultures is only effective at supraphysiological doses. This suggests that NT acts within the hypothalamus as a neuromodulator/neurotransmitter, and perhaps not directly at the pituitary cell. In addition to NT fibers terminating at the external zone of the ME, some NT-positive axon terminals are found in the neurohypophysis. These probably originate in the paraventricular nucleus and play a role in the release of OT and AVP. Finally, the adenohypophysis contains cells that stain NT-positive. It seems unlikely that this material arises from neuronal sources, but it is probably synthesized directly in the pituitary, where it plays a paracrine or autocrine role in the secretion of one or more of the adenohypophyseal hormones.

2.5.2.4. POMC-Derived Peptides. POMC is a large mol-wt precursor protein (265 aa), which in the human (with subtle differences between mammals) is post-translationally cleaved into moieties *ACTH (39 aa), β-lipotropin (β-LPH; 89 aa)* and a *16-K N-terminal fragment* of 76 aa (Fig. 19). Each of these is further cleaved enzymatically to yield: *α-melanophore-stimulating hormone (α-MSH=ACTH$_{1-13}$)*, and *corticotropin-like intermediate-lobe peptide (CLIP=ACTH$_{18-39}$)*. β-LPH is further cleaved into *γ-LPH (β-LPH$_{1-56}$)* and the endogenous opioid *β-endorphin (β-end=β-LPH$_{59-89}$)*. *β-MSH* is a cleavage product of LPH $_{(39-56)}$, and *γ-MSH* is a fragment of the 16k N-terminal peptide. These are widely distributed in the CNS (Table 8).

Aside from the adenohypophysis, ACTH is found in cells of the arcuate nucleus/ME area. The cells are diffusely distributed throughout this area, which extends rostrally to the retrochiasmatic area, caudally to the submammillary region, and dorsally to the area between the ventricular surface and the VMN of the hypothalamus. The fact that hypophysectomy does not influence the amount of ACTH in this area indicates that this activity is *not* a product of the corticotropes of the adenohypophysis. Thus, ACTH is actually formed in the brain. Since ACTH and other peptides are common products of POMC, it is not surprising that β-LPH, α-MSH, β-MSH, and β-END are colocalized with ACTH in these neurons. These neurons give rise to numerous ACTH-positive fibers, which are distributed widely throughout the brain. Within the hypothalamus, ACTH fibers terminate in the anterior, mediobasal, and periventricular areas of the hypothala-

Table 8
Localization of POMC-Derived Peptides

Peptide	*Cell bodies*	*Terminals*	*Presence in portal blood*
ACTH	Arcuate Nucleus, ME Area.	Anterior Hypothalamic Periventricular area dorsomedial nucleus, paraventricular Nucleus, OVLT, ME, preoptic area.	+*
β-LPH	Same as ACTH		ND
γ-MSH	Same as ACTH		+*
β-END	Same as ACTH		+

+ = Yes, in concentrations greater than peripheral blood.
– = No, concentrations the same as or less than peripheral blood.
ND = Not determined.
* = Probably by retrograde blood flow from pituitary gland.

mus. In the periventricular area, the fibers actually penetrate the ependymal lining of the third ventricle. ACTH is measurable in the CSF. ACTH terminals are found in the dorsomedial nucleus, the magnocellular and parvicellular portions of the paraventricular nucleus, and the OVLT. In addition, ACTH terminals are found in the external zone of the ME close to the portal capillaries, as well as within the neurohypophysis. Terminals are also found in the medial preoptic area.

β-LPH cell bodies are also found in the arcuate nucleus/ME area, with fibers projecting to various areas of the brain. In general, ACTH-positive fibers and β-LPH fibers project to the same areas of the brain. β-LPH is also found in the corticotropes of the adenohypophysis. These common distribution patterns are reasonable, considering the common precursor of both.

α-MSH is secreted from the intermediate lobe of the pituitary gland in all vertebrates. Although it has a dramatic skin-coloring effect in poikilotherms, the role of α-MSH in homeotherms is uncertain. There is some suggestion that it may play a role in the secretion of hormones from the adenohypophysis. Within the hypothalamus, α-MSH-positive cell bodies and fibers are found in the same areas of the arcuate nucleus/ME that were described for ACTH. The fibers, for the most part, project to the same areas as the ACTH-positive groups. There is also a second group of α-MSH-positive cells, which are distinct from those in the medial basal hypothalamus. These cells are concentrated in the area between the dorsomedial nucleus and in the fornix and in the lateral hypothalamic area. These cells do not colocalize α-MSH with any other of the POMC-derived peptides, suggesting that the biosynthetic route of α-MSH in these cells may be quite different than that of the mediobasal hypothalamus or the intermediate lobe of the pituitary gland. Fibers from this group project to the caudate-putamen complex, the neocortex and various parts of the hippocampus. As with the POMC-derived peptides, β-END-positive cell bodies are most numerous in the arcuate nucleus/ME area of the hypothalamus, and the course of their efferent projections is similar to ACTH, LPH, and MSH. Similarly, β-END is also found in the intermediate lobe of the pituitary gland. β-END is also found in significant concentrations in hypophyseal portal blood. Thus, β-END qualifies as a neurotransmitter/neuromodulator as well as a neurohormone. Since β-END binds to opiate receptors throughout the nervous system, it has been classified an endogenous opioid.

2.5.2.5 Enkephalins. The opioid peptides are derived from three different precursors. The derivation of β-END from POMC has been described. Smaller opioids known as the enkephalins (ENK) are pentapeptides derived from larger molecules known as *proenkephalins* (Fig. 20). One, proenkephalin A (50 K_d, 267 aa), contains four copies of *methionine-ENK (met-ENK)* and one copy of *leucine-ENK (leu-ENK)*, and one copy each of a met-ENK C-terminal heptapeptide and a met-ENK C-terminal octapeptide. The other, proenkephalin B (also known as prodynorphin) contains three copies of leu-ENK. The

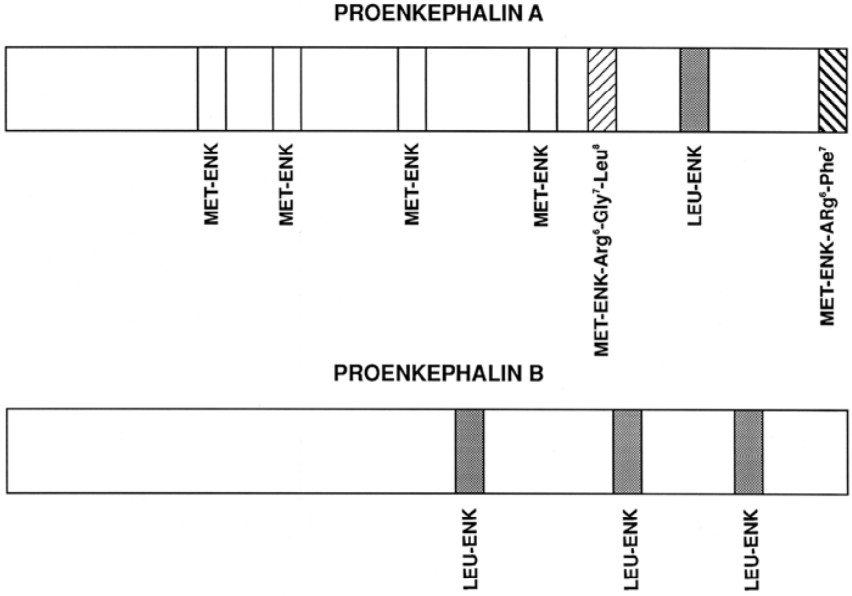

Fig. 20. The structure of proenkephalin A and proenkephalin B. Note the four repeating met-ENK sequences, the single leu-ENK, and each single met-ENK heptapeptide and octapeptide characteristic of proenkephalin A. Proenkephalin B is characterized by three intramolecular leu-ENK sequences.

Table 9
Localization of Enkephalins

Peptide	*Cell bodies*	*Terminals*	*Presence in portal blood*
Met-ENK	Supraoptic nucleus, paraventricular nucleus preoptic area, dorsomedial/ventromedial nuclei.	ME, neurohypophysis.	+
Leu-ENK	Same as Met-ENK.		+

+ = Yes, in concentrations greater than peripheral blood.
– = No, concentrations the same as or less than peripheral blood.

most widely distributed opioid peptides are the ENKs, with met- and leu-ENK found in the same areas. In general, met-ENK is found in higher concentrations than leu-ENK. With regard to the hypothalamus (Table 9), ENK-positive cell bodies are found in the supraoptic and paraventricular nuclei. ENK-positive efferents project from these areas and terminate in the external zone of the ME adjacent to the portal vessels and in the neurohypophysis. Met-ENK is reported to be present in portal blood. In addition, cells in the intermediate lobe of the pituitary gland contain the hepta- and octapeptide of met ENK, but not free met-ENK. In the adenohypophysis, gonadotropes contain all forms of met-ENK. These are not the same gonadotropes that colocalize β-END. Moreover, there is a population of gonadotropes that also contain prodynorphin. The medial preoptic nucleus as well as the dorsomedial and ventromedial hypothalamic nuclei also contain ENK-positive cell bodies. The ENKs appear to regulate pituitary hormone secretion by act-

Table 10
Localization of "Other" Hypothalamic Peptides

Peptide	*Cell bodies*	*Terminals*	*Presence in portal blood*
AII	Paraventricular nucleus, Supraoptic nucleus, adenohypophysis.	ME, neurophypophysis, dorsomedial nucleus.	–
NPY	Arcuate nucleus, ME, dorsomedial nucleus, locus coeruleus.	Medial preoptic area, anterior hypothalamic area, periventricular area, suprachiasmatic, supraoptic, paraventricular, arcuate, and ventromedial nuclei, ME.	+
Galanin	Supraoptic, paraventricular and arcuate nuclei.	ME, neurohypophysis.	+

+ = Yes, in concentrations greater than peripheral blood.
– = No, concentrations the same as or less than peripheral blood.

ing as neuromodulators/neurotransmitters. In the neurohypophysis, ENK inhibits the release of OT and AVP. In the adenohypophysis, ENK inhibits LH and stimulates prolactin, growth hormone, and ACTH secretion by acting within the hypothalamus as a neuromodulator/neurotransmitter. The colocalization of the ENKs with pituitary hormones suggests a paracrine or autocrine role of ENK, but the only direct effect has been shown in the neurohypophysis on the inhibition of OT and AVP.

2.5.2.6. Other Hypothalamic Peptides. Within the past few years, several neuropeptides have been described, on the basis of their neuroanatomical location and pharmacologic studies, as potentially significant regulators of hypothalamic function (Table 10). Although the evidence for their physiological significance is incomplete, they should be considered as potential physiological regulators of hypothalamic function.

One peptide, *angiotensin II (AII)*, plays a role in vasoconstriction, sodium retention, antidiuresis, and drinking behavior through direct actions on peripheral structures such as the adrenal cortex and kidney, an action on the CVOs such as the OVLT, the AP and the SFO, and a direct action on the hypothalamus. There is also physiological evidence that *AII* affects the secretion of LH and prolactin from the adenohypophysis through a neuromodulator/neurotransmitter, neurohumoral and even a paracrine or autocrine role. *AII* is formed by the action of a renal proteolytic enzyme, *renin*, acting upon a peptide produced in the liver, *angiotensinogen*, to form a circulating decapeptide known as *angiotensin I (AI)*. AI, in turn, is cleaved by an *angiotensin-converting enzyme (ACE)* produced in the lungs to form the biologically active octapeptide *AII*. Peripherally, *AII* acts on smooth muscle in arterial walls to promote vasoconstriction and raise blood pressure. Application of AII directly to the CVO also evokes an increase in blood pressure, secretion of AVP and short-latency drinking behavior. The CVOs are outside of the blood–brain barrier, and possess a significant number of AII receptors. AII stimulates the adrenal cortex to secrete aldosterone, the hormone that promotes sodium retention by the nephron. Certain hypothalamic and extra-hypothalamic structures bear *AII* receptors and are sensitive to the application of *AII*. The preoptic area contains a large number of *AII* receptors, and its cells increase their firing rate when *AII* is applied microiontophoretically. These cells mediate the dipsogenic effects of *AII*. The paraventricular nucleus is also sensitive to *AII*. It is not clear how peripheral *AII* gains access to these centers. However, there is abundant evidence for the existence of *AII*-producing elements within the CNS. In fact, *AII*-producing cell bodies are found within the magnocellular cells of the paraventricular nucleus as well as within the supraoptic nucleus. The efferent projections of these cells terminate within the ME as well as within the neurohypophysis. *AII* terminals are also found concentrated in the dorsomedial

nucleus of the hypothalamus and scattered throughout the medial basal hypothalamus. It has been reported that *AII* and AVP are copackaged, and that *AII*, renin, and OT are also copackaged. In the adenohypophysis *AII* has been reported to be packaged in gonadotropes. Taken together, these various locations of *AII*-positive cells and terminals would explain the neuromodulator/neurotransmitter roles (CVO), the neuroendocrine role (supraoptic, paraventricular nuclei; neurohypophysis), and the paracrine/autocrine role (gonadotrope of the adenohypophysis) subserved by *AII*.

Neuropeptide Y (NPY) is a highly conserved 36-aa peptide, which is widely distributed in the CNS in many mammals. Of particular importance to hypothalamic function are the extensive networks of NPY-positive fibers and terminals within the medial preoptic area, the periventricular and anterior hypothalamic areas, the suprachiasmatic, supraoptic and paraventricular nuclei, and the arcuate and ventromedial nuclei as well as the ME. Significant concentrations of NPY are found in hypophyseal portal blood. Within the hypothalamus, NPY-positive cells are distributed in the arcuate nucleus/ME area and in the dorsomedial nucleus. Interestingly, much of the hypothalamic NPY originates from noradrenergic cells outside of the hypothalamus. These cells are found in the lateral reticular medulla, the nucleus tractus solitarius region, the locus coeruleus, and the subcoeruleus. Transection of ascending noradrenergic fibers does not eliminate but substantially decreases NPY immunoreactivity in various hypothalamic areas. NPY has multiple actions within and outside the CNS. Most striking is its effect on the adenohypophysis and on some behaviors. Specifically, NPY plays a role in the regulation of gonadotropin secretion by acting within the hypothalamus—as a neuromodulator/neurotransmitter that affects GnRH secretion and as a neurohormone that affects LH secretion directly. NPY has also been implicated in the control of secretion of ACTH, growth hormone, and prolactin from the pituitary gland. It may act as a neurotransmitter, affecting the secretion of AVP from the neurohypophysis. Moreover, NPY is synthesized in a subpopulation of thyrotropes in the adenohypophysis, suggesting a paracrine/autocrine role. Finally, NPY controls eating behaviors through intrahypothalamic pathways.

Galanin is a highly conserved 29-amino-acid peptide that is widely distributed throughout the central and peripheral nervous systems. Within the hypothalamus, galanin-positive cell bodies are found in the supraoptic and paraventricular nuclei as well as the arcuate nucleus. Dense efferent fibers from these areas terminate in the external and internal layer of the ME as well as in the neurohypophysis. Galanin-like immunoactivity and its message are expressed in somatotropes, lactotropes, and thyrotropes within the adenohypophysis. Expression in thyrotropes and in lactotropes is positively regulated by thyroid hormones and estrogen, respectively. Galanin has been implicated as a neurotransmitter/neuromodulator in the control of growth hormone, ACTH, TSH, LH, and prolactin secretion. It has also been found to colocalize with CRH and GnRH in the hypothalamus. Galanin is secreted from cells of the adenohypophysis and is present in portal blood. Taken together, galanin affects pituitary hormone secretion as a neurotransmitter/neuromodulator, as a neurohormone and as a paracrine/autocrine factor.

Endothelins (ETs) are a family of regulatory peptides with vasoconstrictor activity, originally isolated from incubation media of vascular endothelial cells. One, designated ET-1, is a 21-residue peptide that contains two intramolecular disulphide bonds. Two related peptides, designated ET-2 and ET-3, differ by two and six amino acid residues, respectively. The localization of the ETs in the supraoptic and paraventricular nuclei of the hypothalamus, and the adenohypophysis and neurohypophysis—and the presence of ET-receptors in the hypothalamus as well as the adenohypophysis and neurohypophysis—has prompted active inquiry into the role of the ETs in pituitary hormone secretion. In general, the ETs act within the hypothalamus to enhance LH secretion via stimulation of GnRH. Within the pituitary, they are capable of directly stimulating LH, FSH, TSH, and ACTH secretion. In addition, they are potent inhibitors of prolactin secretion, but exert no action on growth-hormone secretion from the pituitary gland. It is not known whether the ETs are in the portal circulation. These data suggest that the ETs can act as neuromodulators/neurotransmitters, or even act in a paracrine/autocrine manner to affect pituitary hormone secretion.

3. TECHNIQUES FOR STUDYING HYPOTHALAMIC FUNCTION

Many techniques have been used to study hypothalamic function. Below is a partial description of some of the approaches available.

3.1. Sampling Methods

The response to stimuli suspected of involving the hypothalamus are studied at several levels. The end point or dependent variable may be the response itself.

Table 11
Sampling Methods for Studying Hypothalamic Function

Methods	*Use*
Micropunch	Determine amine or peptide content of various areas of the hypothalamus.
Push–pull perfusion	Measure amine or peptide dynamics in CSF.
Microdialysis	Measure amine or peptide dynamics in CSF.
Collection of hypophysial portal or peripheral plasma	Measure neurohormones in portal blood and peripheral plasma.

Thus, one might manipulate the hypothalamus and observe alterations of behaviors, temperature regulation, or pituitary hormone secretion. In addition, the alterations of the biogenic amines and neuropeptides accompanying natural or artificial stimuli may be measured directly. In making these measurements, the investigator is confronted with two problems: a method of collecting the sample that will not impact upon the quantitation of material and reliable measuring tools.

The content of neurotransmitters and neurohormones in hypothalamic tissue can be measured (Table 11). Although there are several *brain microdissection techniques* available, a particularly innovative and useful approach is the *micropunch technique*. This involves the punching from fresh or frozen sections of brain—areas as small as nuclei—for subsequent measurement of content of biogenic amine or neuropeptide. The area is "punched" with needles constructed from stainless steel tubing. The dimensions of the punched area are determined by the size of the needle. The pellet is blown out into a dish or tube for subsequent homogenization and extraction. Using this technique, large numbers of samples can be rapidly processed.

Although the micropunch technique represents a highly useful method for estimating the tissue content of neurotransmitters and neurohormones, its utility is limited by the fact that a single animal can only be sampled at a single point in time. This has been overcome by two related methodologies: *push–pull perfusion* and *microdialysis* (Table 11). Each method has the distinct advantage of multiple sampling over time. To use push–pull perfusion, concentric stainless steel cannulae are implanted so that the tip of the inner cannula is located at the desired site of study (Fig. 21). Artificial CSF lacking the biogenic amines and neuropeptides is "pushed" through the inner cannula and instantaneously "pulled" through the outer cannula into an appropriate receptacle. Assuming the push-pull rates are matched, the "pulled" CSF should be rich in biogenic amines and neuropeptides. The related technique, microdialysis, has been likened to the implantation of an artificial blood vessel in tissue. A probe bearing a small piece of semipermeable dialysis membrane at the end is implanted into the hypothalamus. The end is localized in the area of interest for study. Artificial CSF or saline is pumped through the probe and recovered. Theoretically, amines or peptides will diffuse from the area of higher concentration in the brain to the area of lowest concentration on the probe side of the dialysis tubing. The size-exclusion selectivity of the membrane will determine the size of the molecule diffusing into the probe, and the length of the membrane will determine the amount of tissue sampled.

The concentration of biogenic amines and neuropeptides can even be measured in the microscopic vessels, the hypothalamo–hypophyseal portal vessels connecting the ME with the adenohypophysis (Table 11). Collection of the blood in these vessels free from dilution by peripheral blood involves a complex ventral surgical approach to expose the ME. Once exposed, the stalk is cut and placed inside a polyethylene cannula. Using this procedure, blood is collected from all of the cut portal vessels simultaneously. Although this procedure involves experimental manipulation and collection of portal blood under anesthesia in rats, surgical approaches have been developed to collect portal blood from unanesthetized sheep and monkeys.

3.2. Methods of Quantitating Hypothalamic Function

3.2.1. Assay Techniques

There are two modern assay techniques currently used to measure catecholamines and indolamines in tissue, media, CSF, and blood (Table 12). One technique, the *microradioenzymatic assay*, takes advantage of the fact that the catecholamines and indolamines are methylated in vivo. Dopamine, norepinephrine, and epinephrine are O-methylated in vivo to form metho-

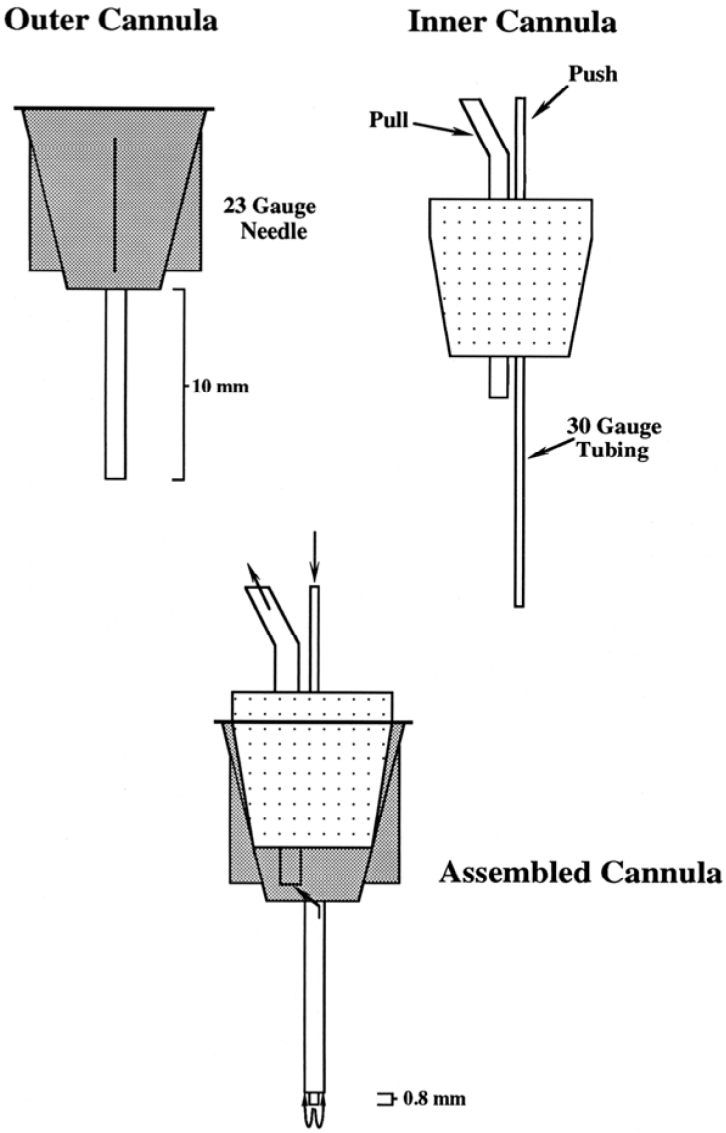

Fig. 21. The structure of the inner, outer, and assembled push–pull perfusion cannula. The arrows indicate the direction of flow through the assembled cannula.

Table 12
Methods of Quantitating Hypothalamic Function

Methods	*Use*
Microradioenzymatic assay	Measure catecholamines or indoleamines.
High-performance liquid chromatography/ electrochemical detection	Measure catecholamines or indoleamines.
In situ voltammetry	Measure catecholamines or indoleamines.
Radioimmunoassay	Measure neuropeptides or pituitary hormones.
Hybridization assay	Measure peptide message.

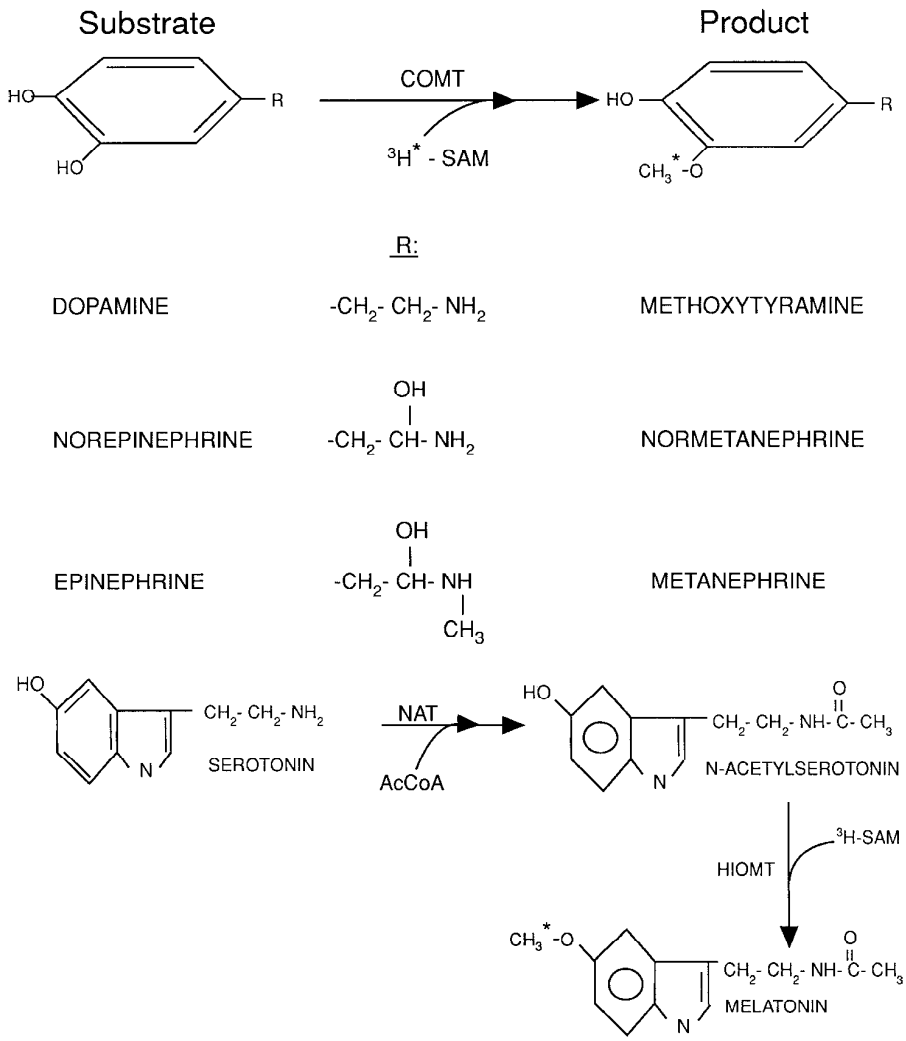

Fig. 22. The critical reactions in the microradioenzymatic assay for catecholamines (dopamine, norepinephrine, and epinephrine) and indoleamines (serotonin). COMT=catecholamine-o-methyl transferase; ^{3}H-SAM=tritiated S-adenosyl methionine; NAT= N-acetyl transferase; AcCoA=Acetyl CoA; HIOMT=hydroxyindole-O-methyl transferase. The amount of the tritiated methylated products (methoxytyramine, normetanephrine, metanephrine, and melatonin) is proportional to the amount of the respective starting substrate (dopamine, norepinephrine, epinephrine, or serotonin). *=position of ^{3}H donated by ^{3}H-SAM.

xytyramine, normetanephrine, and metanephrine, respectively, and serotonin is O-methylated and *N*-acetylated to form melatonin (Fig. 22). Radio-enzymatic assays exploits these metabolic fates by incorporating ^{3}H into the structure of the O-methylated derivatives. In the case of the catecholamines, this is accomplished by using the enzyme catechol *O*-methyl transferase and as methyl donor, ^{3}H-S-adenosyl methionine (^{3}H-SAM). Similarly, the serotonin assay is based upon the ability of hydroxyindole *O*-methyl transferase to catalyze the transfer of the ^{3}H-methyl group of SAM to *N*-acetyl serotonin and thus form melatonin. Since the amount of radiolabeled product is proportional to unlabeled substrate, the isolation of these products by thin-layer chromatography represents a quantitative estimate of substrate. The other modern widespread approach to quantitating the catecholamines is through the use of *high-performance liquid chromatography* coupled with *electrochemical detection (HPLC-EC)* (Table 12). Using this technique, separation of catecholamines is achieved with an analytical column packed with C_{18} reverse-phase material. This material allows resolution of catecholamines, their precursors and metabolites as well as

serotonin and its metabolites. Resolution of sample molecules takes place by their differential interactions with the mobile-phase solvent and the column packing material. Distinct bands of solute form during passage through the column. Resolution of the solutes are controlled by pH, ionic strength, and the nature and concentration of aqueous phase as well as the concentration of the organic components of the mobile organic phase. Quantitation is achieved by eluting the resolved solutes through the electrochemical detector. The potential applied to the detector's cell favors oxidation of the catecholamine. For a given set of operating conditions, the oxidative current is directly proportional to the concentration of electroactive species in solution. Related to the HPLC-EC procedure is a method for determining catecholamine flux in vivo in neural tissue. This procedure, known as *in situ voltammetry*, involves stereotaxic placement of a carbon-based microelectrode. As a potential is applied and increased, the catecholamines in a thin surface adjacent to the electrode are oxidized. The magnitude of the oxidizing current generated is a function of the concentration of electroactive species in solution. The potential at which the current appears is specific for particular catecholamines. Unfortunately, this technique cannot differentiate subtle differences in side-chain groups and thus dopamine, norepinephrine, and epinephrine cannot be adequately differentiated.

Neuropeptide content in various areas of the hypothalamus or concentration in portal blood is routinely measured by *radioimmunoassay (RIA)* (Table 12). Similarly, RIAs are used to measure the hormones of the anterior and posterior pituitary gland that the hypothalamus controls. RIAs are dependent upon the ability of relatively specific antibodies to recognize the unlabeled species of neuropeptide or hormone after it has been labeled with a radioactive tag such as ^{125}I. Since the binding sites on the antibody are the same and are specific, there is a competition between labeled and unlabeled species for that binding site. Thus, the amount of binding of labeled peptide is inversely proportional to the amount of unlabeled peptide with which it competes. The unlabeled peptide would either be varying known amounts of standard or unknown amounts extracted from tissue or blood.

With the advent of modern molecular biological techniques, it is possible to measure the amount of peptide in the hypothalamus, and even to measure the regulation of neuroendocrine peptide gene expression by quantitation of specific messenger RNA. In general, most of the methods for measuring neuroendocrine peptide message are some variant of a *hybridization assay* (Table 12). The basis for the assay is to manipulate the animal in vivo or the cells in vitro with a specific treatment and then to isolate cell nuclei. During the isolation, RNA polymerases remain bound to the genes being transcribed. The nuclei are then incubated in vitro with radiolabeled nucleotide triphosphates, and the polymerases will continue to transcribe the genes for several hundred nucleotides. Thus, the newly synthesized transcripts are being labeled. The critical requirement of this type of assay is the availability of specific cDNA probes for the neuropeptide under study. The specific RNA transcripts are now *hybridized* to the cDNA probes bound to an inert matrix such as nitrocellulose filter, which are subsequently washed and counted in a scintillation counter. The amount of radioactivity counted is proportional to the amount of *specific* mRNA.

3.2.2. Electrophysiology

Electrophysiological studies of the hypothalamus have provided a great deal of information on the firing patterns of hypothalamic neurons. However, this information is most valuable only when the firing patterns are correlated with a hypothalamic-dependent event such as pituitary hormone secretion, or behavioral responses. In general, hormone release from cells or neuropeptide release from axon terminals in the hypothalamus follows an influx of calcium through membrane depolarized during action-potential activity.

Extracellular recordings from hypothalamic nuclei in vivo can be used for topographical or functional identification (Table 13). For example, magnocellular neurons in the paraventricular nucleus can be excited antidromically and identified by electrically stimulating the neurohypophysis. The topographical origin of those neurons terminating in the neurohypophysis can be identified by this method. Similarly, these neurons can be recorded orthodromically after application of a suckling stimulus and correlated with the release of oxytocin into peripheral plasma. Such a relationship suggests, but does not prove, a functional role for the neurons recorded from in control of pituitary hormone secretion.

Several in vitro electrophysiological approaches can be used to study neurotransmitter effects on hypothalamic neurons or effects of hypophysiotropic substances of hypothalamic origin on target cells

Table 13
Electrophysiological Methods for Studying Hypothalamic Function

Methods	*Use*
Extracellular recordings	Topography of neurons.
Hypothalamic slice recordings	Characterize neuronal excitation.
Voltage clamp or current clamp	Neurosecretory mechanisms.

Table 14
Neuroanatomical Methods for Studying Hypothalamic Function

Methods	*Use*
Immunocytochemistry	Visualize location of amines and peptides, activity of neurons.
Tract tracing	Visualize axons.
Autoradiography	Characterize transmitter binding sites, peptide message.

(Table 13). Slice preparations of whole hypothalami allow for introduction of drugs or other factors by superfusion close to the neuron being recorded. Excitable membrane properties of pituitary cells can be studied after application of suspected neurohormones of hypothalamic origin. *Voltage-clamp* or *current-clamp* approaches are used to study the effects of hypophysiotropic substances on the secretory function of pituitary cells (Table 13). These yield valuable information about the identity of ion channels involved in secretion.

3.2.3. Neuroanatomical Approaches

At the present time, *immunocytochemistry* is the favored approach for visualizing neuropeptides in neuronal cell bodies, dendrites, axons, and axon terminals (Table 14). The technique involves saturating histologically prepared sections of hypothalamus with antisera that are specific for the neuropeptide in question. The reaction complex is then treated with an anti-immunoglobulin that has been bonded with an enzyme that will cause a visible precipitate. Alternatively, the antibody can be conjugated with a fluorescent chromogen, which will produce a distinctive fluorescent color.

Localization of peptide in different parts of neurons requires different approaches and produces different information. For example, in order to study neuropeptides in cell bodies, axonal transport must be blocked with agents that disrupt microtubules such as colchicine. Cell-body density can then be estimated. Topographical three-dimensional localization of neuropeptide-containing neurons can be determined. Axons are most difficult to visualize by immunocytochemistry because neuropeptides are transported from the cell body to the nerve terminal by fast axoplasmic flow, and thus not enough material is available for immunostaining. Nerve terminals, however, can be visualized by immunocytochemistry under the light microscope.

Tract-tracing techniques can be employed either alone or in combination with immunocytochemical approaches to determine the path taken by axons of particular neurons (Table 14). Two approaches can be used. Tracts can be visualized from the neuronal-cell body to the axon terminal (anterograde) or from the nerve terminal to the cell body (retrograde). An enzyme—horseradish peroxidase, a glycoprotein capable of catalyzing the oxidation of some chromogens—can be used for both anterograde and retrograde tracings. Fluoro-gold is a fluorochrome that is used specifically for retrograde tracings.

Neurons can also be labeled for activity. The product of the protooncogene c-fos—Fos—can be detected in the nuclei of active neurons by immunocytochemistry (Table 14). Neuronal activity can also be quantitated autoradiographically with the 2-deoxyglucose technique. Active neurons preferentially utilize glucose for oxidative metabolism. 2-deoxy-{D-}^{14}C glucose

Table 15
Methods for Creating Deficits of Hypothalamic Function

Methods	*Use*
Hypophysectomy	Create deficits of neurohypophysial and adenohypophysial hormones and targets for adenohypophysial-releasing hormones.
Stalk transection	Create deficits of neurohypophysial hormones and releasing hormones at their targets.
Surgical lesions	Destroy groups of cells or fibers suspected of involvement in hypothalamic function.
Chemical lesions	Destroy groups of cells which synthesize and secrete specific neurotransmitters or neurohormones.

is injected intravenously, the hypothalami are prepared and sectioned by conventional histological techniques, and the concentration of grains in particular nuclei is evaluated microdensitometrically.

Autoradiography can be used to localize neurotransmitter-binding sites, trace axonal connections, locate sites of steroid receptors, and evaluate neuronal activity. The system to be studied is labeled, brain sections are prepared histologically, the slides are covered with a radiosensitive emulsion, and the emulsion is developed photographically.

Just as there are immunocytochemical techniques to study the localization of neuropeptides, enzymes for catecholamine biosynthesis and neurotransmitter or steroid receptors, there are also immunocytochemical techniques for studying the transcription of the message. The technique in widest use is *in situ hybridization* histochemistry. In this case, specific cDNA is used in place of specific antibody (Table 14). The hybridization of the specific cDNA occurs on the brain section, which is then developed by autoradiographic techniques similar to those previously described. Alternatively, nonradioactive probes are now available that allow visualization of a chromogenic reaction.

Finally, the great advantage of these approaches is that they can be used in combination. For example, one might wish to identify hypothalamic neurons that have steroid receptors. Under these circumstances, it is possible to combine immunocytochemistry with steroid autoradiography.

3.3. Creation of Deficits of Hypothalamic Function

3.3.1. Surgical Manipulations

In general, most surgical manipulations involving the hypothalamus and its targets such as the pituitary gland are performed in order to create deficit symptoms (Table 15).

3.3.1.1. Hypophysectomy. This procedure for removal of the pituitary gland from the sella turcica by a parapharyngeal approach was first described in the rat by Philip Smith in 1927. Since the hypothalamus produces neurohormones that control the adenohypophysis and neurohormones that are released from the neurohypophysis, hypophysectomy creates many, but not all, of the deficits of hypothalamic hypofunction. Although hypophysectomy creates deficits of *all* of the adenohypophyseal and neurohypophyseal hormones, a hypofunctional hypothalamus creates deficits of all of the pituitary hormones *except* prolactin. Whereas prolactin levels in blood are virtually undistinguishable after hypophysectomy, circulating prolactin levels increase as a result of a hypofunctioning hypothalamus. This is caused by the removal of the pituitary lactotrope from the influence of dopamine, the physiological prolactin-inhibiting hormone. It is often difficult to verify completeness of hypophysectomy until postmortem inspection. A further problem associated with hypophysectomy is that it is difficult to prevent partial functional regeneration of the hypothalamo–hypophyseal tract, and thus permanently create deficits in OT and vasopressin secretion. In some cases, the aspirated gland can be *transplanted* to sites distant from the hypothalamus. Deficit symptoms of all the hormones except prolactin persist. When transplanted to the sella turcica to allow revascularization by the hypophyseal portal vessels, the deficit symptoms are reversed. These types of procedures allowed early anatomists to conclude that the critical link between the hypothalamus and adenohypophysis was *neurovascular*. Finally, the stalk ME connecting the hypothalamus with the pituitary gland can be *transected*. This procedure causes disruption of

Table 16
Methods for Stimulating Hypothalamic Function

Methods	*Use*
Natural stimuli	To study the relationship between physiological stimuli and hypothalamic function.
Chemical stimuli	To study the relationship between neurotransmitters and hypothalamic function.
Artificial stimuli	To excite groups of hypothalamic neurons and study the effect on a hypothalamically dependent end point.

the hypothalamo–hypophyseal portal vasculature as well as the hypothalamo–hypophyseal tract. This disruption is permanent if regrowth is prevented by placement of a foil barrier in the transected area. Under these circumstances, the deficit symptoms would be the same as hypophysectomy followed by transplantation to a site that is distant from the sella turcica.

3.3.1.2. Lesions. It order to destroy deep-seated nuclei of the brain in areas such as the hypothalamus, the nuclei must be located with accuracy. This is achieved by placing the head of the subject in a device known as a stereotaxic apparatus, which holds it in a predefined, rigid position. With the aid of a map of the brain (a stereotaxic atlas), as well as anatomical landmarks on the skull and the surface of the underlying brain, focal lesions that destroy discrete anatomical groupings of cell bodies can be placed by sending a current through an electrode. Similarly, fibers of passage may be destroyed by placement of a small knife, which can be manipulated to deafferentiate specific axons that control the hypothalamus.

3.3.2. Chemical Lesions

Selective lesions induced by neurotoxins have become a modern tool to study the role of the hypothalamus (Table 15). They can be either site-selective or transmitter-selective. *Monosodium glutamate* or its more potent analog, *kainic acid*, have site selectivity for neuronal cell bodies and dendrites, sparing fibers of passage and axon terminals in areas outside of the blood/brain barrier such as the arcuate nucleus/ME, the OVLT, and the pre-optic area. Thus, glutamate, kainate, or its less toxic analog, *ibotenic acid*, may be injected systemically and will lesion only those areas. Selectivity is further enhanced by local injections stereotaxically. *Gold thioglucose* will selectively lesion the VMN of the hypothalamus.

Neurotoxic lesions can be made in catecholamine or indolamine neurons. *6-hydroxydopamine* or *6-hydroxydopa* will deplete catecholamines in the brain when injected in the third ventricle. They pass the blood-brain barrier, so their specificity may be restricted by stereotaxic injection in small volumes locally. The indolamine neurotoxins are *5,6-* or *5,7-dihydroxytryptamines*, which are injected intraventricularly or locally.

Cysteamine, 2-mercaptoethylamine, depletes *SS* in the CNS and the periphery. In the CNS, cysteamine depletes *SS* in both cell bodies and axons.

Certain plant lectins, such as *ricin*, are taken up and transported retrogradely along axons to cell bodies, and ultimately kill the cell. Specificity is conferred by coupling an antiserum to the peptide made by the cell so that when the antiserum binds to the peptide, specific cells are killed. Alternatively, the cytotoxic plant lectins can be conjugated to hypophysiotropic peptides in order to selectively kill target cells.

3.4. Stimulation of Hypothalamic Function

There are essentially three approaches to studying excitation of the hypothalamus and its consequences (Table 16). One can study hypothalamic function in response to *natural stimuli*. For example, one can record from specific areas of the hypothalamus or study the activities of specific neuropeptides or amine transmitters in the hypothalamus in response to exteroceptive stimuli such as suckling, mating, volume expansion (hypertonic saline), volume depletion (hemorrhage), or alterations in temperature. The activity of hypothalamic neurons can be studied in response to their natural *chemical stimulators* including neurotransmitters as well as neurotransmitter agonists and antagonists. Moreover, the effects of endogenous peripheral factors such as hormones can be described. Under these circumstances, for example, the activity of estrogen on excitation of hypothalamic neurons involved in sexual behavior can be studied. Hypothalamic neurons can be excited by *artificial means*, and the consequence of their activity can be studied. The usual approach is to stimulate electrically

or electrochemically. In the former case, neurons are excited by electrical depolarization, and in the latter case, depolarization is caused by deposition of iron by the stimulating electrode. Using either of these approaches, one can stimulate an area of the hypothalamus and measure a visceral end point.

3.4.1. The Direct Application of Active Hypothalamic Peptides or Amines to Physiological Targets Reveals Their Physiologic Role

One can study the response of target cells to their physiological affecters. For example, cells of the anterior pituitary gland can be enzymatically dissociated and placed in short-term culture. Hypothalamic peptides can be applied to the cultures by perifusion or by static incubation in monolayer cultures. The release of pituitary hormones into the media can be monitored, and intracellular transduction events can be studied with this approach. Using similar approaches, the receptors for the neuropeptides can be studied.

4. PHYSIOLOGICAL PROCESSES CONTROLLED BY THE HYPOTHALAMUS

4.1. The Hypothalamus Regulates Pituitary Hormone Secretion

4.1.1. General Concepts

4.1.1.1. Characteristics of a Neuroendocrine System. As noted previously, the key feature of a neuroendocrine system is the existence of the neurohemal area; the external zone of the ME, at which neurosecretory axon terminals converge upon a capillary bed that ultimately leads to and affects the secretions of the adenohypophysis. Similarly, neurosecretory axons comprising the hypothalamo–hypophyseal tract terminate on sinusoids in the neurohypophysis, and ultimately secrete their products to the peripheral circulation to affect visceral processes. The axons that terminate in the external zone of the ME secrete release- and release-inhibiting *hormones* into the hypothalamo–hypophyseal portal plasma. In order to meet the definition of release- or release-inhibiting hormones, the substances in portal plasma must meet certain criteria (Table 17): (i) They must be *extractable* from hypothalamic or ME tissue; (ii) They must be present in hypophyseal portal blood in *greater amounts* than in the systemic circulation; (iii) Varying concentrations of a particular release- or release-inhibiting hormone in portal plasma must be *correlated with* varying secretion rates of one (or more) of the anterior pituitary hormones in a variety of experimental conditions; (iv) The suspected hormone should stimulate or inhibit one or more pituitary hormone(s) when administered in vivo or applied to pituitary cells or tissues in vitro; (v) *Inhibitors* that antagonize the actions of the release- or release-inhibiting hormones should block or stimulate anterior pituitary hormone secretion; and (vi) Target cells should have *receptors* for the release or release-inhibiting hormones.

4.1.1.2. Concept of Feedback. The hypothalamo-pituitary-target axes can be characterized as a set of links over which information flows. As information is transmitted from link-to-link, it stimulates or depresses a biological response in the next link, but it also influences the activity of an earlier link. Such an influence is referred to as a *feedback*. In general, there are two types of feedbacks. A *negative feedback* (Fig. 23) is one in which the activity of the downstream link inhibits the activity of one or more upstream links. Conceptually, regulation of room temperature by a thermostatically driven furnace fits this model. The furnace, in response to regulation by a thermostat, raises the temperature of the room to a point that is preset on the thermostat. Once that temperature is achieved, the thermostat senses that level and turns off the furnace. If the thermostat is inoperative, the furnace runs extensively, and the temperature in the room rises to the limits of the furnace. In neuroendocrinology, an example of a negative feedback is the ability of adrenal corticosterone to inhibit CRH secretion into portal blood. A *positive feedback* is one in which the downstream link enhances the activity of one or more upstream links. Conceptually, this is a more difficult mechanism to describe accurately. A voice-activated recording device is perhaps the best example of a positive feedback. The voice begins the recording device. When the voice ceases, the recording device stops. Unlike negative feedback, which is a long-term dampening process, positive feedback is relatively brief and inherently unstable. The feedback signal can be provided by a hormone itself or by a nonhumoral metabolite. The classical example of a positive feedback in neuroendocrinology is the ability of ovarian estradiol to stimulate GnRH secretion into portal blood.

Feedback loops can take several routes (Fig. 24). In the case of the hypothalamo-pituitary-target gland axis, a *long-loop feedback* would be blood-borne from the peripheral target gland to affect the hypothalamus or pituitary. A *short-loop feedback* might be exemplified by a pituitary hormone influencing the secretion of its hypothalamic release- or release-inhibiting fac-

Table 17
Required Characteristics of Neurohormones

1. Activity must be extractable from whole hypothalamus or ME tissue.
2. Concentration in hypophysial portal blood must be greater than systemic circulation.
3. Dynamics in portal plasma must be correlated with dynamics of adenohypophysial hormone secretion.
4. Extracted material must be active in vivo and in vitro.
5. Inhibitors of neurohormones should affect physiological end point.
6. Target cells should have receptors for neurohormones.

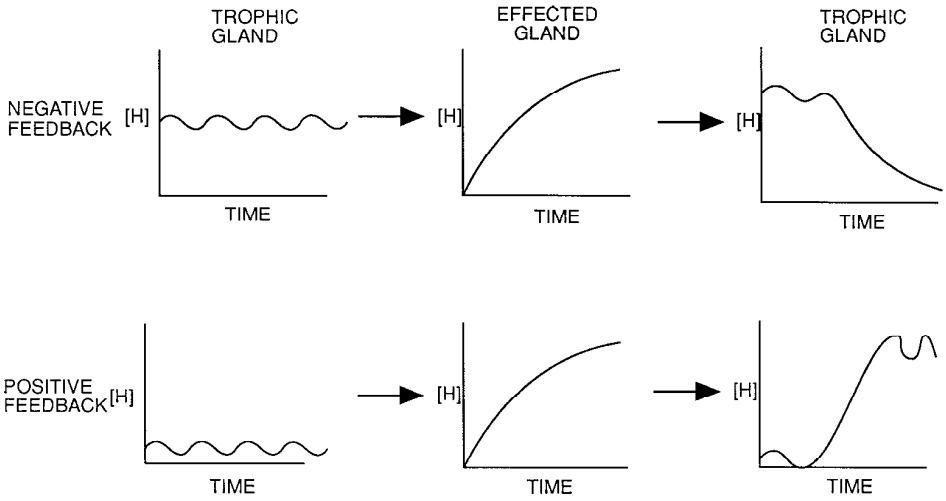

Fig. 23. Diagrammatic representation of a negative feedback (*upper sequence*) and positive feedback (*lower sequence*) in the endocrine system. In a negative-feedback system, the trophic gland (such as the adenohypophysis) secretes a signal (such as TSH), which stimulates the target gland (the thyroid) to secrete its product (thyroid hormone), which in turn feeds back to inhibit TSH secretion. In a positive-feedback system, the trophic gland (the adenohypophysis) secretes a signal at low rates (such as LH), which stimulates the target gland (the ovary) to secrete its product (estradiol), whose increasing secretion rate allows the trophic gland to secrete LH in larger amounts. [H]=hormone concentration in blood.

tor. Finally, an *ultra-short-loop* feedback is represented by a pituitary hormone effecting its own secretion through an autocrine mechanism.

4.1.2. Regulation of the Adenohypophysis by the Hypothalamus

4.1.2.1. Gonadotropes. Overwhelming evidence indicates that the hypothalamic decapeptide known as luteinizing hormone-releasing-hormone (LHRH or GnRH) is the peptide in hypophyseal portal blood that is the physiological humoral stimulator of LH and FSH secretion. Although FSH-releasing activities devoid of LH-releasing activity have been isolated from the hypothalamus, a distinctive FSH-RH has not yet been identified. GnRH regulates LH and FSH secretion from the gonadotropes of the adenohypophysis in both basal and ovulation-inducing "surge" states in female mammals. In rodents, the "surge" center is the medial preoptic area and the basal center is in the medial basal hypothalamus. Males lack a functional "surge" center. The ovarian steroids, estrogen and progesterone, inhibit LH and FSH secretion by acting directly at the gonadotrope, and also at the medial basal hypothalamus to inhibit GnRH release into portal blood. The ovarian steroids also stimulate a preovulatory surge of LH and FSH secretion by acting at the medial preoptic area to stimulate a surge of GnRH into portal blood. This process is presumed to be the result of a noradrenergic mechanism. In rodents, surgical isolation of the medial preoptic area from the medial basal hypo-

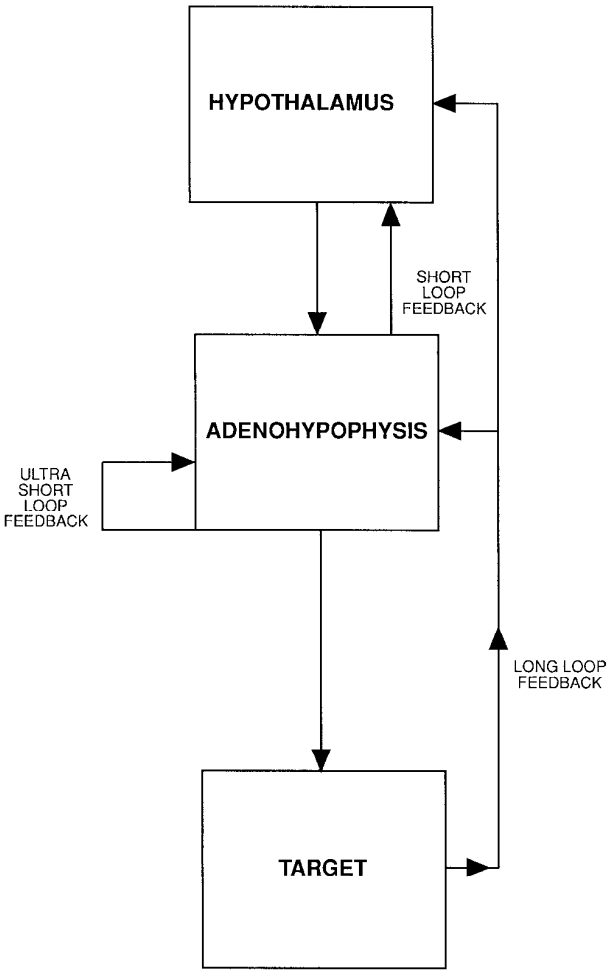

Fig. 24. Diagrammatic representation of feedback loops. In the hypothalamo-pituitary-target gland axis, a long-loop feedback would be blood-borne from the peripheral target gland to effect the hypothalamus or pituitary. A short-loop feedback could be exemplified by a pituitary hormone provoking the secretion of its hypothalamic release- or release-inhibiting hormone. An ultra-short-loop feedback is characterized by a pituitary hormone effecting its own secretion through an autocrine mechanism.

thalamus will prevent a steroid-induced surge of LH secretion. Ovarian and testicular steroids will not induce an LH surge in males. But the gonadal steroids will inhibit LH and FSH secretion, regardless of the sex of the recipient. In primates, there is evidence that the surge center may reside in the basal hypothalamus, and that the ovarian steroid merely sensitizes the gonadotropes to respond to unvarying pulses of GnRH. Monkeys bearing surgically isolated medial basal hypothalami and infused with pulsatile GnRH respond to estradiol with an LH surge. This has led to the concept that the medial basal hypothalamus is a *pulse generator* for GnRH release into portal blood. There is a clear sexual dimorphism of the medial preoptic area: males have a more intensely stained medial preoptic nucleus within this area than females. Such differentiation occurs perinatally. In rodents, castration of males within the first few days of life prevents the appearance of this sexually dimorphic area in adults. Moreover, as adults, neonatally castrated males can respond to an estradiol challenge with a preovulatory-like LH surge. If testosterone is administered to phenotypic female rodents within the first few days of life, they develop the male-type sexual dimorphic nucleus and the male pattern of gonadotropin secretion. Since they lack a surge center, they are anovulatory. Although GnRH is the physiological regulator of LH and FSH secretion, other neuropeptides subserve a similar function, either as neurohormones, neurotransmitters, or neuromodulators. These are listed in Table 18.

4.1.2.2. Lactotrope. As noted earlier, the dominant hypothalamic control over pituitary prolactin secretion is inhibitory. Removal of hypothalamic influence over the adenohypophysis results in an enhanced secretion of prolactin. Thus, stalk transection, destruction of the medial basal hypothalamus or placement of pituitary fragments or cells in culture results in hypersecretion of prolactin. Moreover, in vivo treatment with dopamine antagonists results in hypersecretion of prolactin. Conversely, in vitro treatment with dopamine agonists depresses pituitary prolactin secretion. These data, coupled with the inverse relationship between dopamine levels in portal blood and peripheral blood levels of prolactin, suggest that dopamine is the prolactin release-inhibiting hormone. However, recent studies of the dynamics of prolactin release in response to lowered dopaminergic tone suggest that the lactotrope must also be under the influence of prolactin-*releasing* hormones (PRH) of hypothalamic origin. Although thyrotropin-releasing hormone (TRH) is one of the most widely studied candidates, others (listed in Table 18) have prolactin-releasing properties and may also play a role. Prolactin secretion in response to exteroceptive stimuli such as suckling may thus involve a reduction of dopamine levels in portal blood as well as an increase in the portal-blood concentration of a putative PRH.

4.1.2.3. Thyrotropes. There is little doubt that the tripeptide pyro glu-his-pro-NH_2 is the thyrotropin-releasing hormone (TRH; Table 18). TRH is the physiological stimulator of TSH secretion from the adenohypophysis. The cell bodies with axons that ter-

Table 18
Peptide and Amines that Act Directly on Adenohypophyseal Cells

Cell Type	*Peptide or amine*	*Other peptides or amines*
Gonadotrope	GnRH	VIP
		CCK
		NPY
		Substance P
		Galanin
		Neurotensin
Lactotrope	Dopamine*	TRH
		OT
		VIP
		Angiotensin II
		Somatostatin
		GnRH
Thyrotrope	TRH	Somatostatin*
Corticotrope	CRH	AVP
Somatotrope	GHRH	TRH
	Somatostatin*	

* = inhibit function of cell.

minate upon the external zone of the ME are found primarily in the paraventricular nucleus. In turn, TSH, stimulates thyroid hormone (thyroxine and triiodothyronine) secretion from the thyroid gland. The thyroid hormones, in turn, diminish the release of TSH by lowering the response of the thyrotrope to TRH. This is a classical negative-feedback control system. Removal of the thyroid gland enhances the release of TSH into the peripheral circulation without affecting portal-blood levels of TRH. This further suggests that primary control of TSH secretion by thyroid hormones *does not* reside at the hypothalamus. The hypothalamus provides the drive (TRH), but the thyroid gland negatively regulates (by thyroid hormone) the response of the thyrotrope (TSH) to that drive.

4.1.2.4. Corticotrope. ACTH secretion from the corticotrope is controlled primarily by CRH released into portal blood. ACTH stimulates the release of the steroid hormones of the adrenal cortex, which in turn feed back negatively to inhibit ACTH release by acting at the hypothalamus as well as the adenohypophysis. It is now apparent that arginine vasopressin (AVP) is also a potent ACTH-releasing hormone. Portal blood levels of both AVP and CRH are positively correlated with ACTH-releasing stimuli such as stress. Thus, ACTH release is not caused by the action of a single peptide, but is the result of the actions of a hypothalamic *complex*.

4.1.2.5. Somatotrope. Though the somatotrope is predominantly under the stimulatory influence of hypothalamic GHRH, it is also under the opposing influence of a growth-hormone release-inhibiting hormone, SS. Each neurohumoral peptide affects growth-hormone secretion through distinct receptor sites on the somatotrope, but each plays a reciprocal neuromodulatory role on the other. The SS neurons also directly innervate GHRH neurons with the result of diminishing GHRH release into portal blood, and consequently reducing GH release into the peripheral circulation. Conversely, stimuli known to release growth hormone also suppress release of SS into portal plasma. Feedback control of growth-hormone secretion does not fit the models of classical negative or positive feedback by target endocrine organs. For example, hypoglycemia will stimulate growth-hormone secretion by stimulating the release of GHRH into portal blood. Conversely, hyperglycemia will inhibit growth-hormone secretion by increasing SS and decreasing GHRH levels in portal blood.

4.1.3. The Hypothalamus and Neurohypophyseal Function

4.1.3.1. Mechanism of Secretion of Neurohypophyseal Hormones. The hormone-neurophysin complex (vasopressin-neurophysin II or oxytocin-neurophysin I) are synthesized in cell bodies

of the supraoptic or paraventricular nuclei. The complexes, still undergoing posttranslational processing, are transported down the long axons that comprise the hypothalamo–hypophyseal tract to terminals adjacent to fenestrated capillaries in the neurohypophysis (Fig. 25). Adjacent to the fenestrated capillaries in the neurohypophysis are specialized glial-like cells known as *pituicytes* that regulate the microenvironment of the terminals. During this voyage they are packaged in neurosecretory granules. Once at the axon terminal, the granule membrane fuses with the membrane of the axon terminal and the hormone-neurophysin complex is exocytosed. The process coincides with the arrival of the action potential, which depolarizes the membrane and allows entry of sodium ions. These in turn permit the opening of calcium channels, which plays an unambiguous role in the exocytotic process. After exocytosis of the neurohormone, intracellular calcium is packaged into microvesicles and extruded, and the membrane potential is restored by a sodium-potassium pump. The membranes of the evacuated neurosecretory granules are reformed from the surface of the axon terminal, where they are either packaged into lysosomes and degraded or recycled, usually in areas of nonterminal swelling known as Herring bodies.

4.1.3.2. Stimuli for Secretion of Vasopressin.

The two main stimuli for vasopressin secretion are an increase in osmolality of the plasma and a decrease in plasma volume (Table 19). These can be either interrelated or independent stimuli. Water deprivation causes an increase in plasma osmolality and a diminution of intracellular water. A change in plasma osmolality of as little as 1% is detected by osmoreceptive neurons, which are distinct from the vasopressin magnocellular neurons in the hypothalamus. The osmoreceptive neurons stimulate vasopressin synthesis and release from the magnocellular neurons in the supraoptic area at a threshold of 280 mosM/kg. The osmoreceptor neurons can also stimulate thirst, but with a greater sensitivity of 290 mosM/kg. Vasopressin release is also stimulated by a 5–10% decrease in blood volume, blood pressure, or cardiac output. Hypovolemia is perceived by pressure receptors in the carotid and aortic arch, as well as stretch receptors in the walls of the left atrium, pulmonary veins, and the juxtaglomerular apparatus of the kidney. The afferent impulses of these sensors are carried via the ninth and tenth cranial nerves to the medulla, and then through the midbrain over noradrenergic synapses to the magnocellular vasopressinergic neurons of the supraoptic nucleus. In the absence of any change in pressure, the receptors tonically inhibit vasopressin secretion. With acute volume depletion such as that caused by hemorrhage, noradrenergic inhibitory tone from the medulla to the hypothalamus is decreased, resulting in an increase in the secretion of vasopressin. Volume depletion also stimulates central renin-dependent angiotensin release, which also stimulates vasopressin secretion and thirst.

4.1.3.3. Stimuli for Secretion of Oxytocin.

Suckling is the best-described stimulus for OT secretion (Table 19). As one might expect, the pathways are similar to that of vasopressin. The suckling stimulus is carried over afferent spinal pathways to the medulla and midbrain, and then through cholinergic synapses to the paraventricular nucleus. OT release is pulsatile. Nipple suction by the young leads to synchronized activation of action potentials for 2–4 s in the paraventricular nucleus. From a resting "spontaneous" background of 1–10 spikes/s, these neurons generate a synchronized series of 70–80 action potentials within 3–4 s of application of the stimulus, resulting in the secretion of 0.5–1.0 mI.U. of oxytocin. This is followed by milk ejection from the mammary gland, 12–15 s later. These pulses of neuronal activity occur uniformly every 4–8 min. This characteristic of periodic bursting of action potentials at high frequency appears to be important for OT secretion and consequent milk ejection. The stimuli for OT release during labor appear to be multiple. The activation of cervicovaginal stretch receptors by the growing conceptus is an important signal, and a hormonal background of diminishing placental progesterone and elevated fetal free cortisol act in concert to stimulate OT secretion sufficient to enhance contractions of the uterus. Interestingly, OT secretion has a significant sensory component. In the human female, merely playing with the infant or sensing the cries of a hungry infant will cause milk ejection. In contrast, emotional stress will inhibit the secretion of OT.

4.2. The Hypothalamus Regulates Autonomic Processes

4.2.1. Cardiovascular Function

Independent of the control of blood pressure through the neuroendocrine function of the hypothalamus, the cardiovascular system is influenced by the hypothalamus through the autonomic nervous system (Table 20). These effects are mediated primarily by the sympathetic system through the vagus nerve. Stimulation of the posterior and lateral hypothalamus increases arterial pressure and heart rate, whereas stimulation of the preoptic area decreases heart rate and arterial pres-

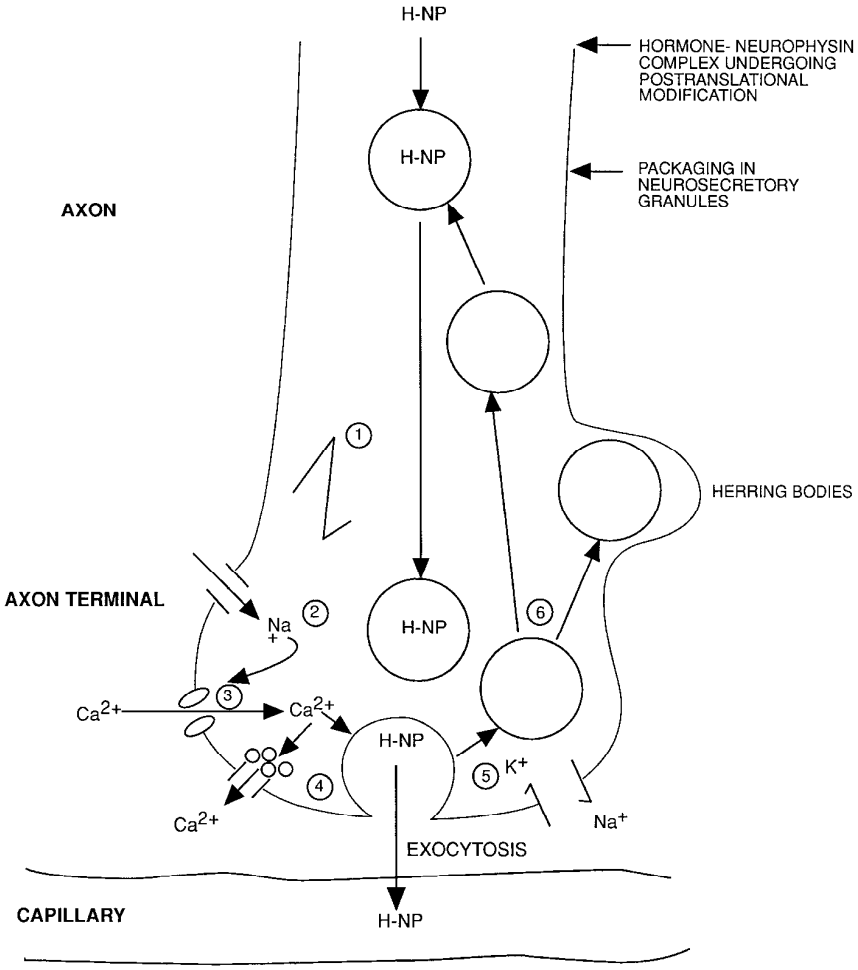

Fig. 25. The mechanism by which the neurohypophyseal peptide-neurophysin complex is axonally transported, processed, packaged, and secreted from the axon terminal. As the hormone neurophysin complex is transported down the axons in the hypothalamo–hypophyseal tract, further posttranslational processing is taking place. The mature complex is then packaged into neurosecretory granules, whose arrival at the axon terminal coincides with the arrival of the action potential (*1*). The membrane of the granule fuses with the axon-terminal membrane, and the product is exocytosed. The action potential is believed to play a role in the process by causing depolarization and entry of sodium (*2*), which in turn allows entry of calcium through specific channels (*3*). Calcium plays a partially understood role in the exocytotic process. The intracellular calcium is then packaged into microvesicles (*4*) and extruded, and the membrane potential is restored by a sodium–potassium pump (*5*). The membranes of the evacuated neurosecretory granules are reformed (*6*), and are either packaged into lysosomes and degraded or recycled in areas of nonterminal swelling known as Herring bodies.

sure. These effects are mediated by the cardiovascular centers in the medulla and pons. Cardiovascular regulation in response to alterations in environmental temperature or defense reactions is also mediated by the hypothalamus. In response to a hot environment, dilation of blood vessels of the skin and constriction of deep visceral vessels occur, and cold exposure induces the opposite responses. These are controlled by the preoptic/anterior hypothalamic areas. The defense reaction that is characterized by cutaneous vasoconstriction and muscular vasodilation is the result of discharge of sympathetic cholinergic vasodilators as well as sympathetic adrenergic excitation. These effects can be induced by selective stimulation of the anterior and posterior hypothalamus.

4.2.2. Thermoregulatory Function

The control of body temperature by the hypothalamus is a classical example of an *integrative* approach to alteration of the *internal milieu*. The hypothalamus oversees *autonomic compensations* such as alterations of blood flow and sweating, *endocrine compensations*

Table 19
Physiological Inputs for Stimulation and Inhibition of Vasopressin and OT Secretion

Hormone	*Stimulation*	*Inhibition*
Vasopressin	↑ Plasma osmolality ↓ Plasma volume ↓ Blood pressure ↓ Cardiac output (α↑,β↓) noradrenergic tone	↓ Plasma osmolality ↑ Plasma volume ↑ Blood pressure ↑ Cardiac output
Oxytocin	Suckling ↑ Activation of cervico–vaginal stretch receptors ↓ Placental progesterone ↑ Fetal cholesterol ↑ Sensory stimulation	Stress

↑ = Increase.
↓ = Decrease.

Table 20
Control of Cardiovascular Function by the Hypothalamus

Area	*Response*
Lateral and posterior hypothalamus	↑ arterial pressure ↑ heart rate
Preoptic area	↓ arterial pressure ↓ heart rate

↑ = increase.
↓ = decrease.

such as metabolism-regulating alterations of thyroid function, and *musculoskeletal compensations* such as shivering, panting and piloerection (Table 21). Temperature regulation by the hypothalamus is also a nonendocrine example of a feedback mechanism. The regulatory system actually collects temperature information from two sources: peripheral sources such as the skin, visceral structures, and spinal cord, and central sources such as thermosensors in the preoptic area/anterior hypothalamus, whose neurons are activated or inactivated by the temperature of the blood bathing them. The hypothalamus bears dual mechanisms for controlling heat dissipation and heat conservation. The heat dissipation centers lie in the preoptic area/anterior hypothalamus, and the heat conservation centers lie in the posterior hypothalamus. Electrical stimulation of the preoptic area/anterior hypothalamus favors dilation of cutaneous blood vessels, panting, and suppression of shivering. All these result in a drop in body temperature. Conversely, electrical stimulation of the posterior hypothalamus leads to cutaneous vasoconstriction, visceral vasodilation, shivering, and a suppression of panting. The metabolic response to temperature alteration also involves the hypothalamus. Exposure to cold enhances the animal's heat-generating metabolic rate by stimulating TRH-activated TSH secretion and subsequent thyroid-hormone secretion. It is clear from recordings of neurons in the preoptic area/anterior hypothalamus that thermosensitive neurons are of two separate types: warm-sensitive and cold-sensitive. Thus, warming of either the skin or hypothalamus results in enhanced firing of warm-sensitive neurons and decreased firing of cold-sensitive neurons. Conversely, cooling of the skin or hypothalamus leads to opposite effects. Thus, these neurons serve to integrate information from the periphery as well as the CNS.

Interestingly, the hypothalamus coordinates voluntary behavioral adjustments to extremes in environmental temperatures sensed at both the hypothalamus and the skin. For example, in both rats and monkeys trained to make behavioral adjustments to a hot environment, local warming of the hypothalamus in the face of normal ambient temperature results in the appropriate behavioral adjustment to warmth. The hypothalamus will also integrate a summation of the responses. Thus when both the hypothalamus and the environment are warmed, the behavioral response is greater than either alone. In a hot environment, cooling of the hypothalamus will completely suppress the behavioral adjustment to elevation of environmental

Table 21
Thermoregulatory Function of the Hypothalamus

Compensation	*Area*	*Response*
Autonomic	Preoptic area	Dilation of cutaneous blood vessels sweating
	Posterior hypothalamus	Vasoconstriction
Musculoskeletal	Preoptic area	Panting Suppression of shivering
	Posterior hypothalamus	Shivering Suppression of panting Piloerection
Endocrine	Preoptic area	Thyroid function

temperature. Thus, the hypothalamus assumes supremacy in the behavioral responses to alteration in temperature. Finally, the hypothalamus mediates the response to pyrogens in pathological states. Body temperature is regulated around a set-point. Substances that allow the temperature to deviate from that set-point—*pyrogens*—can be produced by macrophages in disease state. The preoptic area appears to respond to one such pyrogen, interleukin-1. It has been suggested that the prostaglandins mediate the response to certain pyrogens and act at the preoptic area. Antipyretics such as indomethacin may act by blocking the synthesis of prostaglandins. The brain also contains a nearby *antipyretic area* within the septal nuclei. This area may use the peptide vasopressin. Injection of vasopressin directly into this area counteracts the effects of many known pyrogens. Thus, antipyretics may also act by stimulating the release of vasopressin. Injection of a vasopressin antagonist prevents the antipyretic effects of indomethacin.

4.2.3. Defensive Function

The hypothalamus is also responsible for the preparation of the organism to respond to threatening or stressful situations. The so-called *flight-or-fight* response actually represents an integrated constellation of responses to prepare for stressful situations (Table 22). Many of these responses are directly controlled by the hypothalamus, and others are indirectly controlled by the hypothalamus through its control of the endocrine system. The hypothalamus stimulates a variety of cardiovascular compensations. In response to a perceived threat, blood pressure, heart rate, force of contraction, and rate of cardiac conduction velocity increase. The rate and depth of respiration increases. There is a shift of blood flow from the skin and splanchnic organs to the skeletal muscles, heart, and brain. Metabolic adjustments are made in anticipation of increased energy requirements. These are enhanced glycogenolysis and lipolysis. In addition to the cardiovascular adjustments, there are other autonomic alterations. These would be: mydriasis, ocular accommodation for far vision, contraction of the spleen capsule leading to increased hematocrit, piloerection, inhibition of gastric motility and secretion, contraction of gastrointestinal sphincters, and sweating. Some of these are regulated by the autonomic nervous system directly, and others are controlled by hormones secreted in response to stressful stimuli. Classically, epinephrine is secreted from the adrenal medulla in response to acute stressors. This catecholamine controls many of the metabolic demands of the flight-or-fight response. Additionally, glucocorticoids are secreted from the adrenal cortex in response to stressful stimuli. Secretion of glucocorticoids are controlled by ACTH secreted from the pituitary gland under the influence of hypothalamic CRH and AVP. In long-term stressful situations, this leads to suppression of the immune system. Other hormones whose secretion is stimulated in response to stress (and their putative roles in stress responses) are: β-endorphin (pain perception), vasopressin (renal function), glucagon (carbohydrate mobilization), and prolactin (immune responses). Growth hormone and insulin are typically inhibited during stressful circumstances. Many of these autonomic and hormonal responses are controlled by the anterior and ventromedial hypothalamus.

4.3. Regulation of Behavioral Processes

4.3.1. Ingestive Behavior

4.3.1.1. Hypothalamic Control of Feeding Behavior. The role of the hypothalamus is to coordinate ingestion with parallel neuroendocrine responses and long-term regulation of metabolism and adipos-

Table 22
Components of the Flight-or-Fight Response

1. Increase in: blood pressure, heart rate, force of contraction, rate of conduction velocity.
2. Increase in: rate and depth of respiration.
3. Shift in: blood flow from skin and splanchnic organs to skeletal muscles, heart, and brain.
4. Metabolic adjustments: enhanced glycogenolysis and lipolysis.
5. "Other" autonomic adjustments: mydriasis, accomodation for far vision, contraction of spleen capsule, piloerection, inhibition of gastric motility and secretion, contraction of gastrointestinal sphincters, sweating.

ity. Ingestion during short-term meals is coordinated by brainstem sensory and motor circuits. The nucleus of the solitary tract (NST) relays gustatory and visceral information about ingested food during individual meals by direct and indirect pathways to the hypothalamus. To achieve long-term weight regulation, the appetitive systems of the hypothalamus must also monitor energy storage (in peripheral adiposity) and generate neural signals back to the brainstem to increase or decrease food intake. Body weight is regulated by the balance of energy expenditure (e.g., basal metabolism and locomotor activity) and food intake. Total food intake is the product of number of meals (meal frequency) and meal size. Therefore, body weight can be altered through changes in hunger or appetite (e.g., to change meal initiation and frequency) or sensitivity to satiety signals (e.g., to alter meal size). The regulation of body wt and food intake is achieved by a complex network in the hypothalamus involving multiple transmitter and neuropeptide systems (Fig. 26).

4.3.1.2. Leptin is a Negative-Feedback Adiposity Signal. In order to regulate body weight and long-term energy balance, the hypothalamus must monitor the amount of long-term energy storage in the fat. Although the hypothalamus is sensitive to several other adiposity signals such as insulin, the adipose hormone leptin serves as the primary negative-feedback signal to the brain to regulate fat mass. Leptin is a 127-amino-acid peptide secreted into the circulation from adipocytes; plasma leptin levels are proportional to total body adiposity. Weight loss and food deprivation, which rapidly decrease fat mass and the metabolic rate of adipocytes, lead to a rapid decrease in plasma leptin. Overfeeding, refeeding after fasting, or increased adipose tissue mass increases plasma leptin. Because leptin is a negative-feedback signal, increased leptin secreted by increased fat will reduce food intake; decreased leptin during weight loss causes increased appetite to drive compensatory hyperphagia. Exogenous systemic or central administration of leptin in animals reverses many of the physiological and behavioral correlates of fasting. In normally feeding or obese animals, leptin administration decreases appetite and food intake, and increases metabolic rate, resulting in weight loss. The leptin system is functional in humans, because of a mutation in the leptin gene that blocks leptin synthesis, or a mutation in the leptin receptor that results in functional hypoleptinemia, causes profound obesity and other neuroendocrine deficits. In mutants without leptin signaling, the hypothalamus responds as if the body has no fat reserves of energy: in order to compensate for the apparent starvation state, profound hunger and overeating occurs.

Leptin enters the arcuate nucleus and is transported across the blood–brain barrier to act on hypothalamic neurons that express leptin receptors. The leptin receptor is a member of the cytokine-receptor superfamily, and leptin binding activates Janus Kinasey Signal Transducer and Activator of Transcripton (JAK-STAT) signaling pathways that have both acute effects on neuronal firing rate and long-term effects on gene transcription. Leptin receptors are particularly highly expressed in NPY neurons and POMC neurons of the arcuate nucleus, and to a lesser degree on other cell types of the paraventricular nucleus and lateral hypothalamus. The neurons of the arcuate nucleus comprise interconnected but distinct and opposing pathways regulating food intake: the NPY system and the melanocortin system.

4.3.1.3. NPY System. The NPY neurons of the arcuate nucleus form the major orexigenic or appetite-stimulating system of the hypothalamus. They contain

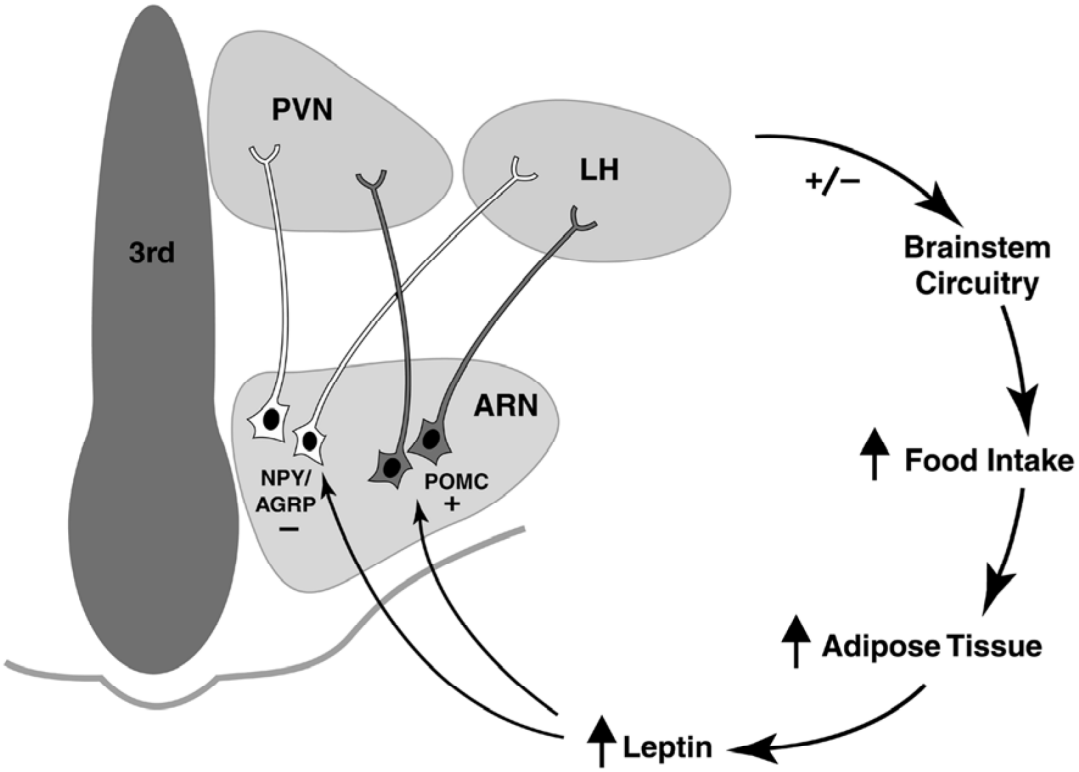

Fig. 26. Hypothalamic integration of ingestive behavior and adiposity. The acute behavior of food intake is largely regulated by neural networks of the pons and medulla. Increased food intake leads to increased fat mass and elevated leptin levels. Leptin provides negative feedback to the hypothalamus to decrease feeding, primarily by decreasing synthesis and release of NPY/AGRP (orexigenic peptides) and increasing synthesis of POMC and thus αMSH (an anorexic peptide) in neurons of the arcuate nucleus (ARN). The ARN neurons project to the paraventricular nucleus (PVN) and the lateral hypothalamus (LH), which in turn modulate brainstem circuitry to decrease food intake and maintain a stable level of adiposity. Food deprivation and reduced fat mass has the opposite effect (to increase food intake), reducing negative feedback by leptin.

the highest concentration of NPY within the brain; they are also unique in their co-expression of agouti-gene-related peptide (AGRP). The primary projection of the NPY neurons is from the arcuate nucleus to the hypothalamic paraventricular nucleus, but they also have long projections to midbrain, pons, and medulla, where they can interact directly with brainstem ingestive circuitry.

Exogenous NPY administered into the third ventricle or the paraventricular nucleus is the most potent orexigen known: nanogram quantities of NPY acting at Y1 and Y5 receptors cause rodents and primates to eat voraciously for hours. Consistent with the NPY system as a positive signal for food intake, food deprivation, and other hunger-inducing treatments cause an increase in NPY mRNA synthesis, peptide synthesis, and NPY release onto the paraventricular nucleus. Furthermore, negative feedback adiposity signals such as leptin and insulin decrease NPY mRNA and peptide levels.

4.3.1.4. Melanocortin System. Intermingled with the NPY neurons of the arcuate nucleus are POMC neurons, which have projections to the paraventricular nucleus and LH that parallel the NPY projections. Although POMC serves as a precursor for several neuropeptides, α-melanocyte-stimulating hormone (αMSH) is the primary product found in the cells of ARN. Although NPY induces appetite, αMSH from POMC neurons acting on MC4 receptors has an opposing satiating effect. In many ways, POMC neurons respond to adiposity signals with a negative effect to balance NPY's positive effects on food intake. Thus, decreased plasma leptin after food deprivation or weight loss decreases POMC mRNA and peptide levels, and increased plasma leptin (e.g., after involuntary overfeeding) increases POMC expression in the

arcuate nucleus in parallel with decreased eating. Injection of αMSH or other MC4 agonists into the hypothalamus reduces food intake; antagonism of the MC4 receptor causes increases in food intake. POMC neurons and MC4 receptors are also present in the brainstem, where they may contribute to local ingestive circuitry. The melanocortin system is critical to human physiology, as mutations in POMC or the MC4 receptor cause obesity in humans.

In an intriguing twist, NPY neurons of the ARN also produce AGRP, an endogenous peptide antagonist of the MC4 receptor. Like NPY, AGRP is a potent orexigen, but it acts by postsynaptically antagonizing αMSH signaling from the POMC neurons to reduce satiety and increase food intake. NPY neurons and POMC neurons have opposing responses to leptin and other adiposity signals, and opposing functional consequences at their target neurons, but the orexigenic NPY/AGRP neurons directly antagonize the satiating effects of the POMC neurons. Thus, the response of the hypothalamus to peripheral adiposity levels is an adjustment of the balance between NPY/AGRP orexigenic and POMC anorexic systems.

4.3.1.5. MCH and Other Peptide Systems. In recent years, a large number of other peptides within the paraventricular nucleus and LH have been implicated in the control of feeding and body weight, including melanin-concentrating hormone (MCH), CRH, galanin, oxytocin, hypocretin/orexin, cocaine-and-amphetamine-regulated-transcript (CART) and TRH (Table 23). These other peptide systems are presumed to be secondary to the major modulatory role of leptin on NPY and POMC neurons of the arcuate nucleus, and can receive input from one or both types of ARN neurons. MCH is a particularly significant member of the secondary systems, because MCH injection into the brain potently induces food intake, and mice lacking MCH are lean, with reduced body fat mass.

4.3.1.6. Serotonin and Norepinephrine. The hypothalamus receives dense innervation of serotonin fibers and norepinephrine fibers from the raphe nuclei and locus ceruleus, respectively, which can modulate the effects of the peptidergic systems. Stimulation of serotonin 5HT-2C receptors reduces food intake by decreasing meal size, and mice lacking 5HT2C receptors show mild obesity in adulthood. Similarly, norepinephrine release into the paraventricular nucleus acting at beta-2 receptors decreases food intake and causes weight loss. Because the agonists of the monoamines are better characterized and are easier than peptidergic compounds to administer systemically, the serotonin and norepinephrine systems are more accessible targets for the pharmacological treatment of obesity than the peptide systems. Thus, the serotonin agonist fenfluramine and the mixed serotonin/norepinephrine reuptake inhibitor subitramine have been used to decrease food intake and induce weight loss in humans.

4.4. Obesity and Pharmacological Control of Appetite

Obesity (defined as a body mass index—weight divided by height squared—of 30 kg/m^2 or greater) is a growing problem in developed countries. Body-fat mass is rapidly increased by easy access to highly palatable, calorie-rich foods. Indeed, by engaging the dopaminergic reward pathways of the limbic system, palatability can override or reset the hypothalamic regulation of body weight. Because it contributes to many other illnesses (e.g., diabetes and cardiovascular disease), obesity is a major public health problem.

In many cases, there may be a genetic contribution to obesity. Mutations that cause obesity or leanness demonstrate the critical role of specific genes in normal behavior and physiology; however, mutations that are homologous to obese rodent mutations are exceedingly rare in humans. The genetic predisposition to obesity in some individuals is probably the result of more subtle interactions of polymorphisms in multiple appetite-regulating genes.

There are many potential points of body-weight regulation that might be targets for therapeutic manipulation. Because of its primary role as an adiposity signal, leptin signaling is an obvious candidate. However, human obesity is accompanied by leptin resistance because plasma levels of leptin are greatly elevated in obese individuals. As mentioned previously, serotonin and norepinephrine are the most accessible factors. Serotonin and norepinephrine receptors are widely distributed in the brain and periphery, and thus, monoamine treatments are usually accompanied by unwanted side-effects. Because the hypothalamus contains unique peptide systems that engage both endogenous appetitive and satiating mechanisms, future treatments may be able to mimic

Table 23
Peptides Involved in the Hypothalamic Regulation of Ingestion

Peptide	*Source*	*Effect*
Leptin	Adipocytes	Anorexic
Val[1]-Pro-Ile-Gln-Lys-Val-Gln-Asp-Asp-Thr-Lys-Thr-Leu-Ile-Lys-Thr-Ile-Val-Thr-Arg-Ile-Asn-Asp-Ile-Ser-His-Thr-Gln-Ser-Val-Ser-Ser-Lys-Gln-Lys-Val-Thr-Gly-Leu-Asp-Phe-Ile-Pro-Gly-Leu-His-Pro-Ile-Leu-Thr-Leu-Ser-Lys-Met-Asp-Gln-Thr-Leu-Ala-Val-Tyr-Gln-Gln-Ile-Leu-Thr-Ser-Met-Pro-Ser-Arg-Asn-Val-Ile-Gln-Ile-Ser-Asn-Asp-Leu-Glu-Asn-Leu-Arg-Asp-Leu-Leu-His-Val-Leu-Ala-Phe-Ser-Lys-Ser-Cys-His-Leu-Pro-Trp-Ala-Ser-Gly-Leu-Glu-Thr-Leu-Asp-Ser-Leu-Gly-Gly-Val-Leu-Glu-Ala-Ser-Gly-Tyr-Ser-Thr-Glu-Val-Val-Ala-Leu-Ser[127]		
AGRP	ARN	Orexigenic (αMSH antagonist)
Leu[1]-Ala-Pro-Met-Glu-Gly-Ile-Arg-Arg-Pro-Asp-Gln-Ala-Leu-Leu-Pro-Glu-Leu-Pro-Gly-Leu-Gly-Leu-Arg-Ala-Pro-Leu-Lys-Lys-Thr-Thr-Ala-Glu-Gln-Ala-Glu-Glu-Asp-Leu-Leu-Gln-Glu-Ala-Gln-Ala-Leu-Ala-Glu-Val-Leu-Asp-Leu-Gln-Asp-Arg-Glu-Pro-Arg-Ser-Ser-Arg-Arg-Cys-Val-Arg-Leu-His-Glu-Ser-Cys-Leu-Gly-Gln-Gln-Val-Pro-Val-Val-Asp-Pro-Cys-Ala-Thr-Cys-Tyr-Cys-Arg-Phe-Phe-Asn-Ala-Phe-Cys-Tyr-Cys-Arg-Lys-Leu-Gly-Thr-Ala-Met-Asn-Pro-Cys-Ser-Arg-Thr[108]		
CART	ARN	Anorexic
Gln[1]-Glu-Asp-Ala-Glu-Leu-Gln-Pro-Arg-Ala-Leu-Asp-Ile-Tyr-Ser-Ala-Val-Asp-Asp-Ala-Ser-His-Glu-Lys-Glu-Leu-Ile-Glu-Ala-Leu-Gln-Glu-Val-Leu-Lys-Lys-Leu-Lys-Ser-Lys-Arg-Val-Pro-Ile-Tyr-Glu-Lys-Lys-Tyr-Gly-Gln-Val-Pro-Met-Cys-Asp-Ala-Gly-Glu-Gln-Cys-Ala-Val-Arg-Lys-Gly-Ala-Arg-Ile-Gly-Lys-Leu-Cys-Asp-Cys-Pro-Arg-Gly-Thr-Ser-Cys-Asn-Ser-Phe-Leu-Leu-Lys-Cys-Leu[89]		
MCH	LH	Orexigenic
Asp[1]-Phe-Asp-Met-Leu-Arg-Cys-Met-Leu-Gly-Arg-Val-Tyr-Arg-Pro-Cys-Trp-Gln-Val[19]		
Hypocretin1/Orexin A	LH	Orexigenic/Wakefulness
Gln[1]-Pro-Leu-Pro-Leu-Cys-Cys-Arg-Gln-Lys-Thr-Cys-Ser-Cys-Arg-Lys-Tyr-Glu-Leu-Leu-His-Gly-Ala-Gly-Asn-His-Ala-Ala-Gly-Ile-Leu-Thr-Leu-Gly[34]		
Hypocretin2/Orexin B	LH	Orexigenic/Wakefulness
Arg-Ser-Gly-Pro-Pro-Gly-Leu-Pro-Gly-Arg-Leu-Pro-Arg-Leu-Leu-Pro-Ala-Ser-Gly-Asn-His-Ala-Ala-Gly-Ile-Leu-Thr-Met-Gly[29]		

See other tables for the sequences of the two major arcuate peptides, orexigenic NPY and anorexic αMSH. Neurons of the arcuate nucleus (ARN) containing NPY/AGRP or POMC/CART are the major targets of leptin; neurons containing MCH, hypocretin/orexin, and other peptides such as galanin, CRH, and TRH are targets of the arcuate neurons. There are two peptide products of the hypocretin/orexin gene; note that they may exert an orexigenic effect by regulating wakefulness and arousal rather than appetite.

or antagonize these endogenous systems specifically and efficaciously.

4.5. Hypothalamic Control of Drinking Behavior

Thirst is regulated by tissue osmolality and vascular volume. These are controlled, in turn, by AVP secreted from magnocellular neurons in the supraoptic nucleus and also by *AII* formed in the plasma as well as the brain. Although the drive for water ingestion is through enhanced tissue osmolality and/or decreased vascular volume sensed by osmoreceptors in the brain and baroreceptors in the brain and periphery, there appears to be a direct effect of hormones acting at the hypothalamus to mediate the behavioral response. The SFO lies near the third ventricle, and has fenestrated capillaries permitting entrance of blood-borne materials. The SFO responds to low levels of AII in the blood and conveys information to the hypothalamus. It is possible that the communication is by way of a neuronally derived *AII* that affects the preoptic area. In addition, the preoptic area receives information from peripheral baroreceptors. Thus, when water ingestion is required, the baroreceptors and *AII* stimulate the preoptic area, which in turn activates other areas of the brain to begin drinking.

The drive for termination of drinking is less understood. However, it is clear that cessation of drinking is not merely the absence of the baroreceptor and osmoreceptor-initiating signal.

4.6. Sexual Behavior

The circumscribed behaviors leading to pregnancy and propagation of the species depend on the interaction of the gonads and the hypothalamus. In subprimate mammals, these events are driven by a heightened period of female sexual receptivity known as *estrus*, which coincides with the availability of a potentially fertilizable egg in the oviduct. Since these female mammals have reproductive cycles characterized by a heightened receptivity, the cycles are referred to as *estrous cycles* (noun:estrus; adjective:estrous). A similar coincidence of gamete availability is not discretely defined in most primates. Since primate cycles are overtly characterized by a period of breakdown of the lining and blood vessels of the uterus known as *menses*, these are referred to as *menstrual cycles*. Obviously, sexual receptivity is best studied in mammals that overtly display the behavior at discrete periods. For this reason, the rat is the most widely studied model of hypothalamic control of sexual behavior.

4.6.1. Hypothalamic Control of Sexual Behavior in Females

Sexual receptivity can be quantitated in female rats by a *lordosis quotient*, or *LQ*. Lordosis is the process by which the female arches her back, deflects her tail, and stands rigid to allow mounting and intromission by the male. The LQ is the number of times this event takes place divided by the number of attempts at mounting by the male multiplied by 100. Although the effects of various hormones on sexual behavior are species-specific, the common hormone that regulates most sexual behaviors is the ovarian hormone *estrogen* (Table 24). Estrogen receptors are present in the areas of the hypothalamus known to control sexual receptivity. Estrogen secretion is highest when sexual receptivity is increased. Although estrogen, by itself, will enhance sexual receptivity, sexual receptivity is greatest when both estrogen and *progesterone* secretion is highest. Progesterone, by itself, exerts little effect on sexual receptivity. Only in an estrogen-primed animal will progesterone further enhance sexual receptivity. Estrogens act by stimulating progesterone receptors in areas of the hypothalamus known to control sexual receptivity. Prolonged exposure to progesterone (as in pregnancy) causes a downregulation of progesterone receptors in the hypothalamus, and subsequently a decrease in sexual receptivity. Four parts of the nervous system have been shown to play a role in the control of female sexual behavior: *the forebrain*, *the ventromedial hypothalamic nucleus (VMN)*, *the midbrain central gray*, and *the lower brainstem and spinal cord*. Within the hypothalamus, lesions of the VMN depress sexual behavior in response to estrogen and progesterone. The VMN bears receptors for the ovarian steroids. This hypothalamic nucleus is believed to modulate the intensity or interpretation of sexually related sensory input. Estrogen receptors are also localized in the midbrain central grey. Neurons from both the VMN and spinal cord project to the midbrain central grey. The spinal projection transmits tactile information provided by the male's mounting required for the induction of lordosis. The neurotransmitter control of female sexual behavior is well-described, but cannot be reduced to participation by a single transmitter. Among the catecholamines, ascending noradrenergic fibers from the locus coeruleus regulate lordosis behavior by acting upon α_1-noradrenergic receptors in the medial preoptic area and VMN. Norepinephrine may act in these areas by modulating progesterone receptors. Dopamine, however, does not play a role in lordosis behavior, but appears to modulate proceptive behaviors such as ear wiggling, hopping, or darting. Acetylcholine plays a role in the facilitation of lordosis behavior through estrogen. This steroid not only increases the activity of choline acetyltransferase, and increases the activity of acetylcholine receptors in the VMN. Moreover, acetylcholine applied directly to the medial preoptic area or VMN increases lordosis behavior, and acetylcholine antagonists applied to these same areas abolish or attenuate lordotic behavior. Among the indolamines, serotonin appears to play an inhibitory role in sexual receptivity. Inhibition of serotonin synthesis excites lordosis behavior, and thus it has been suggested that serotonin is a *sexual satiety* neurotransmitter. A similar role has been proposed for gamma-aminobutyric acid (GABA). Some of the hypothalamic peptides that serve a neuroendocrine role in regulating the pituitary gland also serve a neurotransmitter role in regulating sexual behaviors in the female. The best example is GnRH. When GnRH is applied directly to the hypothalamus of ovariectomized female rats receiving an ineffective dose of estradiol, lordosis behavior is exhibited. This suggests that GnRH both stimulates LH secretion and consequent ovulation and subsequent sexual behavior in the female. In addition, not

Table 24
Hypothalamic Control of Sexual Behavior in Females

Chemical mediator	*Site of action*	*Effect*
Estrogen	Ventromedial nucleus Midbrain central gray	Increase LQ*
Progesterone	Ventromedial nucleus Preoptic area	Increase LQ response to estrogen
Norepinephrine	Ventromedial nucleus Preoptic area	Modulate progesterone receptors
Acetylcholine	Ventromedial nucleus Medial preoptic area	Modulate LQ response to steroids
Serotonin		Inhibit sexual behavior
GnRH	Midbrain central gray	Heighten response to estradiol
Prolactin	Midbrain central gray	Heighten response to estradiol

*LQ = lordosis quotient.

only do preoptic GnRH neurons project to the arcuate nucleus/ME area and subsequently release the peptide into portal blood to bathe the adenohypophysis, but they also project to the midbrain central grey, the site mediating sexual receptivity. GnRH applied to the midbrain cenral grey stimulates sexual receptivity, and GnRH antisera applied to this area depresses sexual receptivity. Aside from neuropeptides, pituitary hormones themselves may play a role in sexual receptivity. Indeed, prolactin applied directly to the MCG enhances sexual receptivity in rats receiving a low dose of estradiol. Conversely, pharmacologic depression of prolactin secretion at the time of anticipated onset of estrus depresses the magnitude of sexual receptivity. Finally, pituitary hormones are released in response to the mating stimulus. It is well-established that prolactin is released from the adenohypophysis of rodents in response to excitation at the uterine cervix by the act of mating, which is transmitted to the hypothalamus through spinal pathways. It has been shown that the mating stimulus acts at the hypothalamus by lowering tuberoinfundibular dopaminergic tone, which subsequently leads to the release of prolactin. Prolactin, in turn, activates the corpora lutea to maintain progesterone secretion, which maintains the subsequent pregnancy. The other pituitary hormone released in response to the mating stimulus is OT. Once again, the stimulus is transmitted over spinal pathways to enhance the activity of magnocellular neurons in the paraventricular nucleus. It has been suggested that once OT is released in response to the mating stimulus, it acts to enhance uterine contractions and thus favor sperm transport from the site of deposition at the mouth of the cervix to the site of fertilization at the oviduct. The lone weakness in this theory is that rather than enhancing contractions of the uterus from the cervical toward the ovarian direction, the contractions are enhanced in the ovarian–cervical direction. This process would appear to retard sperm transport at best.

4.6.2. Hypothalamic Control of Sexual Behavior in Males

The sexual behavior of male rodents is characterized as having both *motivational* and *consummatory* components (Table 25). Motivational are those behaviors required to gain access to the female in heat. Consummatory behaviors are those required for copulation. These would include mounting, erection, intromission, and ejaculation. Stereotypical male sexual behaviors are provoked by testosterone secreted from the Leydig cells of the testis. Testosterone controls male sexual behavior through two mechanisms. In peripheral tissues, testosterone is converted to dihydrotestosterone (DHT). DHT is responsible for stimulating sensory receptors, and thus may play a role in penile erection. Testosterone acts on the preoptic area of the hypothalamus to integrate the various consummatory components of male sexual behavior. The amygdala controls the motivational components of male sexual behavior. This function of estrogen appears to have been aromatized from testosterone intraneuronally. Among the catecholamines, dopam-

Table 25
Hypothalamic Control of Sexual Behaviors in Males

Chemical mediator	*Site of action*	*Effect*
Testosterone	Preoptic area	Control consummatory behaviors.
Estradiol	Amygdala	Control motivational behaviors.
Mesolimbic dopamine	Amydala	Control motivational behaviors.
Incertohypothalamic	Preoptic area	Control consummatory behaviors.
GnRH	Preoptic area	Control consummatory behaviors.
Endorphins	Preoptic area	Inhibit consummatory behaviors.

ine from mesolimbic neurons appears to be the neurotransmitter that controls the motivational component of male sexual behavior. The incertohypothalamic dopaminergic system appears to be responsible for the consummatory component. Neuropeptides have been shown to modulate both motivational and consummatory behaviors. GnRH appears to act within the preoptic area to control consummatory behaviors. Endorphin neurons projecting from the amygdala to the preoptic area appear to have the opposite effects, they inhibit many consummatory behaviors. Other peptides that have been implicated in male sexual behaviors include substance P, NPY, α-melanophore-stimulating hormone and oxytocin. However, the physiological significance of their role has not been fully determined. As mentioned earlier, the preoptic area of the hypothalamus is sexually dimorphic, which is reflected in the pattern of LH secretion from the adenohypophysis. The dimorphic nature of the hypothalamus is also reflected in stereotypical male or female sexual behaviors. Just as males who are deprived of testosterone neonatally present a female cyclic pattern of LH secretion when challenged with estrogen as adult, so they will also present the typical female receptive lordotic pattern in response to estrogen and an aggressive male. Conversely, a female treated neonatally with testosterone will show the non-cyclic pattern of LH secretion as an adult and, if treated with testosterone as adult, will mount females in heat. Some of this sexual differentiation occurs in utero but in rodents most of it is determined neonatally. Thus, all hypothalami develop potentially as functionally female, and the differentiating event is the presence of androgen prenatally or neonatally.

4.7. Maternal Behavior

The hypothalamus is also intimately involved in mediating maternal behaviors stimulated by both ovarian and pituitary hormones (Table 26). Again, the rodent model is the most frequently studied. There are essentially four components of maternal behavior in the rat. *Nestbuilding* is the first behavioral sign. Late in pregnancy, the rat will gather bedding and any other materials available, and prepare a nest in which she can deliver, nurse, and care for the young. She designs this to be the center of all her activities while the young are present. After the pups are born, the dam spends a large amount of time *licking* the neonates for the purpose of cleaning. The typical behavior rodents share with all mammals is assumption of a *nursing* posture to allow the hungry young access to the mammary glands for retrieval of milk. Finally, as the nursing young mature, they tend to leave the convenience and safety of the mother's nest. The nursing mother then spends much time *retrieving* the young to the nest. The hormonal drive for the onset of maternal behavior actually occurs during the prepartum period (Table 26). By supplying foster pups late in pregnancy, the development of these behaviors can be characterized. The signal appears to be the gradual decline in progesterone secretion by the placenta, coupled with the increase in ovarian estrogen secretion as the time of parturition approaches. The prepartum period can therefore be envisioned as a period of *hormonal priming*. Maternal behaviors are not only the result of the combined actions of estradiol in the face of the withdrawal of progesterone, but they also influenced by adenohypophyseal (or perhaps even neural) prolactin. OT may also play a role. Estrogen exerts its effect on maternal behavior largely through an action at the medial preoptic area. Much of the action of estrogen at the medial preoptic area is through stimulation of estrogen receptors. In general, throughout pregnancy estrogen receptors are much greater in the preoptic area than in the entire hypothalamus. On the last day of pregnancy, estrogen receptors in the rest of the hypothalamus rise to levels equivalent to those of the preoptic area. In addition to stimulating parental

Table 26
Hypothalamic Control of Maternal Behavior

Chemical mediator	*Site of action*	*Effect*
Estrogen after decline of progesterone	Medial preoptic area	Stimulate maternal behaviors.
Prolactin	Preoptic area	Stimulate maternal behaviors in estrogen-primed rats.
OT	Ventromedial nucleus	Stimulate maternal behaviors in estrogen-primed rats.

behaviors directly, estrogens also stimulate prolactin secretion. Moreover, it is the withdrawal of progesterone at the end of pregnancy that allows prolactin to exert its actions on the mammary gland to initiate and maintain lactation. Prolactin, when secreted after parturition, has been implicated in the control of maternal behaviors. Indeed, hypophysectomy or treatment with the DA agonist bromocryptine, will prevent many of the components of maternal behavior. In contrast, the infusion of prolactin directly into the preoptic area or into the CSF through the third ventricle stimulates maternal behaviors in estrogen-primed female rats. Thus, prolactin can act upon cells in the preoptic area as well as the circumventricular organs. Since prolactin is a large polypeptide, it is unlikely that it can cross the blood-brain barrier to affect neural structures. There are essentially three possibilities from the route prolactin may take to affect neural structures and subsequently maternal behavior. One is that pituitary prolactin arrives at the hypothalamus by *retrograde blood flow* through the portal circulation. Alternatively, it has been shown that circulating prolactin has access to the CSF and brain through a receptor transport system located in the choroid plexes of the lateral, third, and fourth ventricles. Finally, the most recent evidence indicates that specific areas of the hypothalamus contain prolactin mRNA leading to the suggestion that prolactin is synthesized in these areas distinct from pituitary prolactin. Since estrogen can enhance brain and CSF levels of prolactin in *hypophysectomized rats*, it has been suggested that estrogen stimulates central prolactin synthesis and that centrally prolactin may enhance the sensitivity of estrogen-sensitive cells in the hypothalamus that regulate maternal behavior. Among the hypothalamic peptides, OT has been shown to promote maternal behavior when injected into the CSF of estrogen-treated rats. OT is ineffective when injected peripherally. Moreover, an OT antagonist is effective in delaying maternal behavior when injected centrally. Destruction of the paraventricular nucleus, the source of OT, also modifies maternal behaviors. The effects of OT are correlated with the appearance of OT cell-membrane receptors in areas of the brain known to mediate maternal behavior. These include the VMN of the hypothalamus, the bed nucleus of the stria terminalis, the anterior olfactory nucleus, and the central nucleus of the amygdala.

4.8. Emotional Behaviors

It has long been appreciated that the hypothalamus participates in emotional responses. For example, electrical stimulation of the lateral hypothalamus of cats results in many of the somatic and autonomic characteristics of *anger* such as piloerection, pupillary constriction, arching of the back, raising of the tail, and increased blood pressure. Similar rage-like responses can be elicited by decortication or merely separating the hypothalamus from the cortex. The anger that is elicited is referred to as *sham rage*. Such animals respond to seemingly innocuous stimulation with a multitude of aggressive responses. The hypothalamus appears to act as an integrating center for these responses.

4.9. Regulation of Rhythmic Events

4.9.1. Types of Rhythms

Rhythms (Fig. 27) are characterized by their *period* (the time needed to complete one cycle), *frequency* (number of cycles per unit time), *phase* (points of reference on a time scale), and *amplitude* (the magnitude of variation from the mean). Biological rhythms are endogenous and self-sustaining. The only external cues may be provided by *Zeitgebers* or time-givers such as lighting periodicity in some rhythms. Biological rhythms fall into one of four categories based on their period (Table 27): *circadian* (approximately one day and thus driven by zeitgeber), *ultradian* (less than one day and of much greater frequency such as heart

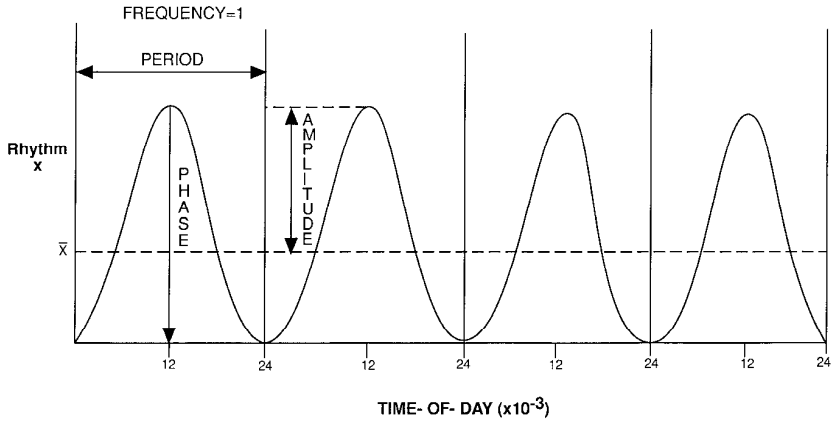

Fig. 27. Parameters of a *rhythm* (x) over four complete cycles of any *zeitgeiber*. For purposes of this example, imagine that the zeitgeber is the lighting periodicity of an artificial 24-h day with daylight lasting from 7 a.m. to 7 a.m. The *period* is the time to complete one cycle of the rhythm. In this example the period is 24 h. The *frequency* is the number of cycles per unit of time. Here the frequency of rhythm x is one (per day). The *phase* is the maximum of the rhythm in reference to a time scale such as that provided by the lighting periodicity or a clock. The *amplitude* is the deviation from the mean of the rhythm, $\bar{x}$.

Table 27
Categories of Biological Rhythms

Rhythm	*Approximate period*	*Example*
Ultradian	Much less than 24 h	Respiration, heart rate
Circadian	Approx 24 h	Corticosterone rhythm
Infradian	Greater than 24 h but much less than 365 d	Menstrual cycles
Circannual	Seasonal, approx 365 d	Hibernation

beat or respiration), *circannual* (greater than one day and usually synchronized with seasonal events, such as seasonal fat deposition) and *infradian* (greater than one day but shorter than a year, such as menstrual or estrous cycles).

4.9.2. Role of the Hypothalamus in Biological Rhythms

We have already described the role of hypothalamic neurohormones and neurotransmitters in infradian rhythms characterized by the menstrual and estrous cycles. Indeed, the cyclic release of luteinizing hormone every 28 d in the human female involves participation of parts of the hypothalamus, ranging from the most rostral to the most caudal boundaries. In rodents, it is particularly useful to appreciate the multifaceted roles of various areas of the hypothalamus regulating the various rhythms that comprise the estrous cycle. For example, it is well-known that GnRH neurons in the preoptic area respond to estrogen secreted every 4–5 d by secreting the peptide into portal blood and consequently release a large bolus of LH into peripheral plasma. Thus the preoptic area controls an infradian rhythm. The preoptic area also controls a circadian rhythm. Again, in rodents, ovariectomy and estrogen replacement results in not just a single preovulatory-like surge of LH secretion, but a surge at the same time on each day for the next several days. Shifting the lighting phase will shift the time of the occurrence of each surge an equivalent amount. Thus, the lighting periodicity is the zeitgeber and the biological event is a true circadian rhythm. It is well-known that hypothalamic circadian rhythms require the participation of a timing device or *clock* within the hypothalamus. This role is served by the *suprachiasmatic nucleus* (SCN) of the hypothalamus. Destruction of the SCN will result in the inability of the rat to transduce the lighting periodicity. A circadian rhythm

generalizable to virtually all mammals is the adrenal corticosterone rhythm. In response to entrainment by lighting periodicity (rodents) or activity rhythms (man), corticosterone levels in the blood begin to increase and reach peak magnitudes at the same time each day. This process has been shown to be driven by pituitary ACTH and hypothalamic CRH as described previously. In rodents, the stimulus is the onset of darkness, whereas in man it is the onset of activity or wakefulness cycles. It is quite clear that the secretion of most pituitary hormones is not merely biphasic (basal and surge pattern), but is actually pulsatile, and that blood levels of hormone at any time represent the summation of an ultradian pattern of hormone secretion from the cell. Such a rhythm is probably the result of the activity of a *pulse generator* within the hypothalamus, regulating neurohormone secretion into hypophyseal portal blood. A pulsatile ultradian rhythm of LH secretion is revealed in ovariectomized rats and monkeys. Measurement of multiple-unit activity in the medial basal hypothalamus of monkeys reveals that the pulsatile pattern of LH secretion coincides with spikes of multiple-unit activity in the medial basal hypothalamus. This implies that the pulse generator for GnRH and subsequent LH secretion (at least in monkeys) resides within the medial basal hypothalamus. Annual or circannual rhythms are not exclusively linked to the hypothalamus. Annual behavioral rhythms are of two types. Type I annual rhythms are dependent upon the environment, and type II are dependent upon an endogenous biological clock. Type I rhythms are generally photoperiodic driven because they require transduction of seasonal changes in day length. For example, as day length shortens during the late summer through early fall (short days) voles reduce their food intake and their gonads involute. Under long days of spring, food intake and gonadal weights return to normal. Type II annual rhythms are those that *free run*, (e.g., require no environmental input and thus persist under constant environmental conditions). European starlings store fat prior to their demanding spring migration. Under constant light, temperature, and food availability, the rhythm of fat deposition persists. Each animal free runs with a period of 1 yr, and eventually become desynchronized under constant environmental conditions. We have already mentioned that photoperiodic time measurement involves the SCN of the hypothalamus. Fibers from the retina of the eye terminate within the SCN. It is over this *retinohypothalamic tract* that the lighting periodicity is transduced. Efferent fibers from the SCN terminate in the paraventricular nucleus, which in turn sends efferent fibers via the medial forebrain bundle to the spinal cord, which terminates upon the *intermediolateral cell column*. Processes from these cells synapse in the *superior cervical ganglion of the sympathetic chain*. Postganglionic noradrenergic fibers from this area then project to and innervate the *pineal gland*. Through this pathway, the SCN generates a circadian rhythm in the pineal hormone *melatonin*, which is synchronized to the light-dark cycle. Melatonin is produced in greatest amounts during dark phases of the cycle, and appears to be most important in mediating the effects of annual rhythms. The testes of male hamsters are most competent to produce sperm and normal levels of testosterone during the long days of summer. Pinealectomy prevents the loss of competency when animals are placed in the abbreviated illumination of short days. However, if pinealectomized hamsters receive long melatonin pulses (signaling short days or long nights) the testes regress independently of the environmental photoperiod. There appears to be a strain difference in the sensitivity to melatonin. In addition, not all mammals are dependent upon the pineal gland for generation of biological rhythms. For example, the rat, a photoperiodic mammal, has normally timed ovulation-inducing surges of LH release from the pituitary gland when pinealectomized. In those animals responsive to melatonin, the sites in the CNS and periphery where melatonin acts to regulate biological rhythms are unclear. Although there are marked species differences, the anterior hypothalamus, the SCN and the adenohypophysis apear to be candidate sites.

5. CONCLUSION

By now, the neuroscience student must appreciate a point made in the Introduction to this chapter. The hypothalamus does not exert control over one process exclusive of other processes. Thus, the hypothalamus plays an *integrative role* in adapting the organism to demands placed upon it by its environment. Because of its multifaceted role in allowing the organism to respond to the environment, the hypothalamus can be appropriately characterized as an organ that most uniquely ensures the perpetuation of the species.

SELECTED READING

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