

**BASIC NEUROENDOCRINOLOGY  
AND THE ANATOMY OF THE HYPOTHALAMUS**

Donald K. Clifton

Department of Obstetrics and Gynecology, and  
Population Center for Research in Reproduction  
University of Washington  
Seattle, Washington  
USA

Correspondence: Donald K. Clifton, Ph. D.  
Department of Obstetrics and Gynecology, RH-20  
University of Washington  
Seattle, WA 98195

Telephone: 206-685-1854  
Fax: 206-543-3915  
e-mail: don@u.washington.edu

## INTRODUCTION

The human pituitary gland lies snugly and securely nestled at the base of the brain in a boney pouch known as the sella turcica. This arrangement provides the pituitary with a great deal of protection from physical assaults and, until relatively recently, has also effectively deterred the curious from discovering the secrets of its function. Hovering over the pituitary like a broody hen over her only chick lies a ventromedial area of the brain called the hypothalamus (Fig. 1). The proximity of these two organs accurately reflects a functional relationship between them; however, it is not safe to conclude, on the basis of their small size, that the pituitary and hypothalamus are relatively unimportant. The pituitary gland operates as an endocrine transducer whereby the brain, working through the hypothalamus, controls a number of important body functions. A defect in either pituitary or hypothalamic regulatory mechanisms can seriously impact the well-being of an individual.

Neuroendocrinology is the study of how the endocrine and nervous systems interact. The scope of neuroendocrinology is broad, and much of it deals with trying to understand how the brain controls pituitary hormone secretion. The purpose of this chapter is to provide a brief overview of hypothalamic neuroendocrinology, leaving the details for elaboration in subsequent chapters.

## PITUITARY HORMONES

It has only been over the last century that scientists have begun to unravel the mysteries that have been locked up within the sella turcica since the creation of man. Modern interest in the pituitary gland and speculation that it might be an endocrine organ can be traced to the last decade of the 19th century. Through the first few decades of the 20th century, spurred on by the early work of Harvey Cushing, a number of important functions became identified with the pituitary gland including growth, metabolism, reproduction, lactation, blood pressure, water retention and uterine contraction. By the early 1930's work to identify the hormones secreted by the pituitary began in earnest, but it wasn't until the mid 1960's that most of these hormones were finally isolated and characterized. The human pituitary is now recognized to secrete eight major hormones of known function which can briefly be summarized as follows:

- The gonadotropins, *luteinizing hormone* (LH) and *follicle-stimulating hormone* (FSH), act at the gonads to regulate reproduction and stimulate sex steroid secretion. LH and FSH participate in ovarian follicular development, ovulation and corpus luteum formation in the female. In the male they work together to promote sperm production by the testis.
- *Prolactin* acts along with gonadal steroids to stimulate breast development and milk production. Although prolactin is also secreted in the male, its physiological role (if any) in men is not known.

- *Growth hormone* (GH) acts at various sites throughout the body to regulate growth and metabolism. GH also stimulates the release of insulin-like growth factor I (IGF-1) which mediates many of the actions of GH.
- *Thyroid-stimulating hormone* (TSH) participates in the regulation of metabolism by acting at the thyroid gland to stimulate iodine uptake and control the release of thyroid hormones.
- *Adrenocorticotrophic hormone* (ACTH) acts at the adrenal cortex to regulate the synthesis and release of corticosteroids and also acts directly on liver and fat tissue to regulate fatty acid metabolism.
- *Vasopressin* (VP), also known as *antidiuretic hormone* (ADH), regulates blood volume by acting at the distal tubule of the kidney to control water reabsorption.
- *Oxytocin* performs two primary functions in the female. First, it acts at the uterus to stimulate contractions during parturition. Second, it acts at the mammary gland to stimulate milk let down. Like prolactin, its function in the male is unknown.

## NEUROSECRETION

The concept that neurons synthesize hormones and secrete them into the circulation was first proposed by Ernst Scharrer in 1928 based on neuroanatomical observations that he had made while studying teleosts (1). At first this idea was discredited by many of Scharrer's colleagues who were convinced that he was misinterpreting pathological artifacts. Undaunted by their colleagues' lack of enthusiasm, Ernst and his wife Berta championed this concept for over 20 years before it finally became generally accepted within the scientific community. Neurosecretion, as this process is now known, is the foundation upon which the modern field of neuroendocrinology now rests.

A neurosecretory neuron is basically like any other neuron, except that its axon terminates in conjunction with a fenestrated capillary rather than making a synaptic connection with another neuron. The hormones that neurosecretory neurons synthesize are usually peptides that can be found acting as neurotransmitters elsewhere within the nervous system, but when produced for neurosecretion are released into the circulation where they travel to act at some distant target site. Neurosecretory neurons range in size and shape from the large magnocellular types located in the supraoptic and paraventricular nuclei to the small parvocellular types found scattered throughout the hypothalamus and basal forebrain.

## ANATOMY OF THE NEUROENDOCRINE SYSTEM

### *Pituitary*

The pituitary (or hypophysis) can be divided into two distinct parts (Fig. 2). The posterior lobe of the pituitary is formed from an extension of nervous tissue from the hypothalamus.

For this reason, the posterior pituitary is also called the neurohypophysis, the pars nervosa, and the neural lobe. The part of the neural extension that forms the bridge between the hypothalamus and the neurohypophysis is referred to as the infundibular stalk. At the top of the stalk, at the interface between the hypothalamus and the pituitary is a region called the median eminence.

The anterior pituitary (or adenohypophysis), which is derived embryologically from the ectoderm of the primitive mouth known as Rathke's pouch, wraps itself partly around and clings tightly to the neural lobe. Sandwiched between the two, but actually more a part of the adenohypophysis is a region of tissue known as the intermediate lobe or pars intermedia. The anterior lobe is further divided into the upper region called the pars tuberalis and the lower region called the pars distalis (Fig. 2).

Oxytocin and vasopressin are the two hormones that are secreted by the posterior pituitary. They are synthesized by neurosecretory neurons in the hypothalamus and are delivered via axonal transport to the neurohypophysis where they are stored in preparation for release (Fig. 3). The axons of vasopressin- and oxytocin-synthesizing neurons terminate in the vicinity of a rich capillary plexus. In contrast to the capillaries found in most of the central nervous system which are generally impermeable to neuropeptides, capillaries within the neurohypophysis are fenestrated, allowing oxytocin and vasopressin to be readily taken up into the circulation.

Unlike the posterior pituitary, the anterior pituitary does not appear to receive any direct innervation from the hypothalamus. The secretory cells of the adenohypophysis are concentrated primarily in the pars distalis (Fig. 3). For the most part, each of the adenohypophyseal hormones is synthesized and secreted by its own particular cell type. Growth hormone, prolactin, ACTH and TSH are derived from somatotropes, lactotropes, corticotropes, and thyrotropes, respectively. The exceptions are LH and FSH which are produced and released by gonadotropes. Gonadotropes, and to a lesser extent, thyrotropes, are not limited to the pars distalis but are found scattered throughout the pars tuberalis as well. Besides these well-characterized secretory cell types, the pars tuberalis also contains cells that appear to synthesize and secrete a product (or products) whose chemical structure and physiological significance remains unknown (2).

### ***Pituitary Circulatory System***

The way that blood flows to and through the pituitary gland is a very important issue and still somewhat controversial. The neural lobe is supplied by the inferior hypophyseal artery that empties into a rich capillary bed surrounding neurosecretory nerve terminals (Fig. 4). Venous drainage from the neurohypophysis occurs through the inferior hypophyseal vein into the cavernous sinus. The importance of this system beyond supplying basic metabolic requirements is highlighted by the observation that regional neurohypophyseal blood flow is about eight times greater than cortical blood flow (3).

Blood supply to the adenohypophysis is somewhat more circuitous. Linking the anterior pituitary to the hypothalamus is a portal circulatory system that consists of 1) a primary

capillary plexus spanning the pars tuberalis and the median eminence, 2) a secondary plexus in the pars distalis, and 3) a system of portal veins connecting the two (Fig. 4). When this system was first described by Popa and Fielding in 1930 (4), they reported that blood travels from the anterior lobe to the median eminence. While this would provide an efficient method for the pituitary to deliver hormones to the hypothalamus, it is now clear that the principle direction of flow is just the opposite. The primary plexus receives its blood mainly from the superior hypophyseal artery. There does not appear to be a separate arterial supply to the pars distalis; all of the blood that reaches the pars distalis comes by way of the portal system. While it is frequently assumed that the primary venous drainage from the anterior lobe is through direct venous connections with the cavernous sinus, this may not be the case. Bergland and Page have observed that this route appears to be too limited to effectively drain all of the blood arriving from the median eminence (5). They suggest that short portal vessels connecting the infundibular stalk to the adenohypophysis also carry venous effluent from the pars distalis into the neurohypophyseal capillary plexus. The complicated arrangement of capillaries and portal vessels in the pituitary and median eminence make it possible that under some circumstances retrograde blood flow may occur, resulting in the delivery of pituitary hormones to the hypothalamus in high concentrations. Whether or not this ever occurs is still a matter of speculation and considerable debate.

### ***Hypothalamus***

The hypothalamus lies buried deep within the brain at the base of the diencephalon, directly under the thalamus. The wide range of physiological functions that it serves is reflected in the diverse population of neuronal cell types it contains and in the unique and complex interconnections that exist between these cells. Because the hypothalamus is so inaccessible and does not have the same highly-structured cellular organization that is found in other regions of the brain such as the cortex, hippocampus and cerebellum, it is a difficult structure to study.

The formal boundaries of the hypothalamus are as follows (6). Dorsally, it is bounded by the hypothalamic sulcus which separates it from the thalamus and laterally it is bounded by the internal capsule and the subthalamus. The lamina terminalis (the boundary between the diencephalon and telencephalon) forms the rostral boundary and an imaginary line extending from the caudal aspect of the mammillary bodies to the posterior commissure is taken as the caudal boundary. The ventral aspect of the hypothalamus is limited by the floor of the diencephalon. These boundaries, particularly the rostral and caudal ones, appear to be somewhat arbitrary; actually, the hypothalamus merges seamlessly with the telencephalic septal region at its rostral extent and the central gray and tegmentum of the midbrain at its caudal extent.

As viewed from its ventral surface, the hypothalamus extends from the mammillary bodies to the optic chiasm; the region between these two landmarks is known as the tuber cinereum. Protruding from the midline of tuber cinereum is the infundibular stalk. As mentioned earlier, the dorsal portion of the stalk that interfaces with the hypothalamus is

called the median eminence. The lateral eminence is formed by swellings of the tuber cinereum on either side of the median eminence.

The median eminence is divided into two major zones. The external zone is situated on its ventral surface and contains the short capillary loops that form the major part of the primary portal plexus. This is an area that contains a variety of different types of nerve endings, some glial cells and ependymal processes, but few neuronal cell bodies. The external zone is rich with neuroactive substances; a wide variety of neurotransmitters are concentrated in this region, including many types of neuropeptides, biogenic amines, and other classical transmitters (7). The internal zone of the median eminence is sandwiched between the external zone and the floor of the third ventricle. It contains the fiber tracts that connect the hypothalamus to the neurohypophysis, some long capillary loops of the primary portal plexus, and the ependymal lining of the ventricle. The ependymal cells in the median eminence connect the CSF of the third ventricle to the portal capillary system (8); however, the physiological significance of these connections is still a matter of speculation.

The lateral hypothalamus contains only a few neuronal perikarya along with the axons of a major fiber tract known as the medial forebrain bundle which forms a major input/output pathway for the hypothalamus. Most hypothalamic neurons aggregate in clusters that are located primarily in the medial part of the hypothalamus, alongside the third ventricle. The following is a description of those hypothalamic areas that are particularly important neuroendocrine regulatory centers (Fig. 5):

- The *arcuate nucleus* is situated ventrally on along the side of the third ventricle in the region of the infundibular recess. It is strategically located directly above the median eminence and is involved in the regulation of a variety of neuroendocrine functions. This nucleus comprises one of the few regions in the brain that is outside of the blood-brain barrier, and therefore can be influenced by blood-borne factors that cannot penetrate most other areas of the brain.
- The *ventromedial nucleus* (VMN) lies dorsal to the arcuate nucleus. It acts as a major relay station for extrahypothalamic connections and is involved in the regulation of feeding and sexual behavior.
- The *paraventricular* (PVN) and *supraoptic nuclei* (SO) contain the magnocellular perikarya that project to the neurohypophysis; they synthesize and secrete the posterior pituitary hormones. The supraoptic nucleus, as its name implies, is situated immediately dorsolateral to the very caudal part of the optic chiasm and the initial segment of the optic tract. The paraventricular nucleus is located in the anterior hypothalamic region rostral and dorsal to the ventromedial nucleus. The parvocellular part of this nucleus contains neurosecretory neurons that regulate anterior pituitary function.
- The *periventricular nucleus* (PeN) forms a thin lining along the side of the third ventricle from its most rostral extent at the preoptic recess to the rostral arcuate

nucleus. Neurosecretory cells within this nucleus participate in the regulation of growth hormone secretion from the anterior pituitary.

- The *medial preoptic area* (mPOA) is a rather diffuse region that straddles the lamina terminalis and therefore is not strictly contained entirely within the hypothalamus. This area plays an important role in the control sexual behavior and the secretion of anterior pituitary hormones.
- The *suprachiasmatic nucleus* (SCN) is a small nucleus that sits on top of the optic chiasm and actually receives direct inputs from fibers traveling from the retina through the chiasm. This nucleus plays an important role in the coordination and regulation of circadian rhythms.

## ANTERIOR PITUITARY HORMONE REGULATION

Anterior pituitary hormones, which are synthesized and secreted by endocrine cells located primarily in the pars distalis of the adenohypophysis, are regulated both by the hypothalamus and by circulating hormones secreted by peripheral endocrine glands such as the gonad, adrenal cortex, and thyroid. The mechanism by which the hypothalamus regulates the anterior pituitary was the subject of much debate during the first half of this century. However, it is now clear that this mechanism involves a neurovascular link between the brain and the pituitary (Fig. 6). Neurosecretory neurons in the hypothalamus synthesize neurochemicals known as releasing and inhibiting hormones. These neurons extend their axonal processes to the median eminence where they terminate in the vicinity of capillaries associated with the primary portal plexus. Releasing and inhibiting hormones secreted from neurosecretory nerve terminals are picked up by the portal system and carried through the portal veins into the secondary plexus in the pars distalis. After diffusing out of the portal plexus, these hypothalamic hormones act on the appropriate endocrine cells through specific cell-surface receptors.

Most of the releasing and inhibiting hormones are peptides, although it appears that other neuroactive substances can also perform this role under some circumstances. The peptides are generally derived from larger precursor molecules that are cleaved and processed to form the mature hormone. It has become clear that these hypothalamic hormones not only serve to regulate pituitary hormone release, but most of them also act as neurotransmitters elsewhere in the nervous system. In fact, it appears that besides projecting to the median eminence, some neurosecretory neurons have axon collaterals that form synapses with other neurons both inside and outside of the hypothalamus. Furthermore, many of the neurosecretory neurons that synthesize releasing and inhibiting hormones also synthesize and secrete one or more additional neurotransmitters.

### ***Gonadotropin-Releasing Hormone***

One of the first releasing hormones to be identified was gonadotropin releasing hormone (GnRH, also known as luteinizing hormone-releasing hormone or LHRH). This decapeptide stimulates the release of both LH and FSH from the anterior pituitary. How

one releasing hormone can independently regulate the release of two pituitary hormones is a matter of debate. GnRH-containing neurons originate in the olfactory placode from which they migrate during embryonic development into preoptic area and arcuate nucleus (9, 10). (In rodents the migration does not extend beyond the anterior hypothalamic region.) Axons of the GnRH neurons that regulate gonadotropin secretion project to the lateral external zone of the median eminence. A wide variety of neurotransmitters have been found in terminals making synaptic contacts with GnRH neurons including neuropeptide Y, substance P, neurotensin, galanin, proopiomelanocortin, gamma-aminobutyric acid, serotonin, norepinephrine, dopamine, vasopressin, corticotropin-releasing hormone (CRH), and GnRH itself (11). Besides synthesizing GnRH, some GnRH neurons in female rats also produce galanin which is a neuropeptide that appears to play an important role in the generation of the preovulatory LH surge (12, 13). Whether or not the coexpression of galanin with GnRH also occurs in female primates is still under investigation. Some GnRH neurons also appear to synthesize delta sleep-inducing peptide (14), but its role in these cells is unknown.

### ***Somatostatin***

GH is controlled by both releasing and inhibiting hormones. The inhibiting hormone is a 14-amino acid peptide called somatostatin (SS). SS-containing neurons are found throughout the brain where they perform a variety of functions as neuropeptide transmitters. SS is also found in a number of peripheral tissues including the pancreas and gut. The SS neurons that are directly involved in the regulation of GH secretion are located primarily in the PeN. These neurons receive synaptic inputs from cells containing neuropeptide Y, gamma-aminobutyric acid, serotonin, corticotropin-releasing hormone, growth hormone-releasing hormone and SS (11).

### ***Growth Hormone-Releasing Hormone***

Growth hormone-releasing hormone (GHRH) is a 44-amino acid peptide produced by neurons in the arcuate nucleus. In addition to GHRH, some of these neurons also produce galanin (15, 16), neurotensin (15, 17), and probably gamma-aminobutyric acid (15) and dopamine (15, 18) as well. Other neurons that make synaptic contact with GHRH neurons include those that contain substance P, met-enkephalin, catecholamine, thyrotropin-releasing hormone, SS, and GHRH itself (11).

### ***Thyrotropin-Releasing Hormone***

Thyrotropin-releasing hormone (TRH) is another neuropeptide that is widespread throughout the brain and peripheral nervous system. The TRH neurons that are responsible for the regulation of TSH release from the anterior pituitary are located primarily in the parvocellular part of the PVN. Because TRH is a peptide that contains only three amino acids, it was originally thought that it was synthesized enzymatically. However, it is now clear that it is cleaved from a larger precursor molecule like other hypothalamic hormones. TRH neurons receive synaptic connections from terminals that

contain catecholamines (19), serotonin (20), neuropeptide Y (21), corticotropin-releasing hormone (22) and TRH (22).

### ***Corticotropin-Releasing Hormone***

The release of ACTH from the pituitary is controlled by a 41-amino acid peptide known as corticotropin-releasing hormone (CRH). CRH neurons form synaptic contacts with most of the other neurosecretory cells of the hypothalamus including SS, GnRH, TRH and other CRH neurons. Although CRH cells are found elsewhere in the brain, the cells that are responsible for controlling ACTH secretion reside in the parvocellular PVN. In addition to CRH, these cells coproduce-produce a variety of neuropeptides including vasopressin (23, 24), oxytocin (25), neurotensin (26), and enkephalin (26, 27). Paraventricular CRH neurons are innervated by cells containing SS, TRH, CRH, catecholamines, serotonin, acetylcholine, gamma-aminobutyric acid, neuropeptide Y, proopiomelanocortin, oxytocin and VP (11, 22).

### ***Prolactin Regulation***

Unlike the other five pituitary hormones, the control of prolactin is primarily through an inhibitory factor. When the pituitary is transplanted to some other site so that it is no longer under the influence of the hypothalamus, LH, FSH, GH, TSH, and ACTH secretion fall to very low levels, but prolactin secretion increases dramatically. Also unlike other pituitary hormones, prolactin does not appear to have a specific peptide releasing or inhibiting hormone. The primary factor regulating prolactin release is most likely dopamine, a catecholamine neurotransmitter. Dopamine (DA) is found primarily in the substantia nigra, but the DA that controls prolactin secretion comes from neurons that are located in the arcuate nucleus/median eminence region. DA is released into the portal circulation and exerts a strong inhibitory influence on prolactin secretion from the anterior pituitary. Besides DA, other neurochemicals that are present in the median eminence appear to influence prolactin secretion as well (28-30).

## **CONTROL OF PITUITARY HORMONE SECRETION**

### ***Rhythms***

Pituitary hormones are secreted in a rhythmic fashion. These rhythms have periods that can be as long as a year or as short as several minutes. Circannual rhythms, which occur on a yearly basis, are used by animals to adjust to seasonal changes in their environment. On a much faster time scale, body functions are synchronized to the time of day by circadian (daily) rhythms in hormone secretion. In addition, most hormones are released

in discrete pulses that occur anywhere from once every few minutes to several times a day. The secretory patterns associated with these pulses are referred to as ultradian rhythms. The secretion patterns of pituitary hormones usually consist of a complex combination of several different rhythms.

### *Ultradian Rhythms*

The pulsatile release of pituitary hormones was first described over twenty years ago, but we are still trying to understand how and why these pulses are generated. Although some anterior pituitary cells might exhibit low level pulsatile activity (31), most ultradian rhythms are thought to be a pituitary response to the pulsatile secretion of releasing and inhibiting hormones from the hypothalamus. The release of discrete pulses of hypothalamic hormones into the portal circulation not only suggests that the corresponding neurosecretory neurons undergo periodic bursts of activity, but also implies that these cells act together in a coordinated fashion. It is not clear whether ultradian pulses derive from some intrinsic rhythmic property of neurosecretory neurons, or if they are driven by some common external source. So far, no external driving source has been identified for any of the neurosecretory systems. However, there is some evidence, at least for the GnRH system, that neurosecretory neurons are intrinsically rhythmic (32). If this is the basis for the ultradian rhythm in GnRH secretion, then there must be a network of interconnections between GnRH neurons to synchronize their activity. As detailed in the previous section, there is evidence for synaptic interconnections among the neurosecretory neurons of each of the hypothalamic releasing/inhibiting hormone systems.

The physiological significance of pulsatile hormone release is not completely known, but a number of hypotheses have been advanced. First, it appears that at least some hormones are more effective when secreted in a pulsatile fashion. For example, the efficacy of GH can be enhanced by administering it in discrete pulses (33). Second, in some cases pulsatile secretion appears to be required to maintain the response of the target organ to the hormone. The monkey gonadotrope seems to be particularly sensitive to the patterning of GnRH secretion. When GnRH is given continuously to monkeys, LH levels initially increase, but then fall to very low levels; the pituitary response to GnRH can be restored by shifting from a continuous to a pulsatile mode of administration (34). Third, it appears that the frequency of hormone administration can convey additional information to the target cell. In fact this is one way that a single hormone could differentially control two different processes, with one process sensitive to low frequency stimulation and the other process sensitive to high frequency stimulation (35).

### *Circadian/Circannual Rhythms*

Circadian rhythms are present in most physiological systems, so it is not surprising that they play an important role within the hypothalamic neuroendocrine system. In fact, many of the body's circadian rhythms are secondary to 24-hour variations in neuroendocrine activity. There are two major types of circadian rhythms. One is driven by environmental cues such as light or temperature. This type of rhythm ceases immediately when the animal is placed in a constant environment. The other type of rhythm is

generated by an endogenous oscillator that is synchronized to an environmental cue. In the absence of the environmental cue, the oscillator free runs at its natural frequency which is usually slightly longer or slightly shorter than 24 hours. Therefore, the rhythm persists but is no longer coupled to the time of day.

One of the most important circadian oscillators in the mammal resides in the suprachiasmatic nucleus. While the mechanisms responsible for the generation of rhythmic activity in the SCN remain unknown, it does appear that it is not dependent on action potentials. Schwartz and coworkers found that infusion of tetrodotoxin (a blocker of sodium-dependent action potentials) into the SCN blocked the free-running circadian activity rhythm in rats; however, the rhythm reappeared *in phase with the previous rhythm* after the infusion was terminated (36). This suggests that although the expression of the rhythm is action potential-dependent, the oscillator continues to function in the absence of classical neurotransmission. Perhaps a transcriptional oscillator analogous to the *per* gene oscillator in the *Drosophila* nervous system is responsible for maintaining rhythmicity within individual neurons of the SCN (37). This would mean that the responsibility for generating a circadian rhythm is distributed across cells in the nucleus and would help to explain why it is possible to destroy any part of the SCN without completely disrupting circadian activity.

The SCN receives direct input from the eye through axon collaterals from the optic chiasm. It also receives indirect retinal input that is relayed through ventral lateral geniculate nucleus and intergeniculate leaflet. These connections are involved in synchronizing the rhythm to the light cycle. The SCN makes efferent connections with neurons throughout the hypothalamus and limbic system. These connections appear to be important for maintaining rhythms in the secretion of ACTH, prolactin, LH and FSH as well as other important body functions such as motor activity, feeding, drinking, sexual behavior, and core temperature regulation (38).

The pineal gland is also involved in the maintenance of circadian activity and plays an important role in the regulation of circannual rhythms in photoperiodic species. This small organ, which is situated near the posterior commissure just above the third ventricle, has also been referred to as the “third eye”. This is because in lower vertebrates it lies close to the surface of the brain and receives photic stimulation through the skull. However, in the mammal it is situated too deep in the brain to function in this manner. Instead, the mammalian pineal receives visual cues through a very circuitous route. Information is transmitted from the SCN through the PVN and spinal cord to the superior cervical ganglion where it is relayed to the pineal via sympathetic fibers (Fig. 7). The primary hormone of the pineal is melatonin which it produces on a circadian basis, with the greatest rate of synthesis occurring at night. Because melatonin is lipophilic, its secretion into the circulation is thought to occur passively as a function of its synthesis. Once in the circulation it travels back to the brain where it diffuses across the blood-brain barrier to act on receptors located in the pars tuberalis, SCN, and at other sites scattered throughout the hypothalamus to exert its effects on the neuroendocrine system.

## ***Feedback***

The hypothalamus and pituitary work together to stimulate target tissues throughout the body. Most target tissues respond to this stimulation by releasing their own hormones into the circulation. Target tissue hormones are used to relay information about the status of the target tissue back to the hypothalamus and pituitary (Fig 8). This feedback arrangement allows hypothalamic neurosecretory neurons and endocrine cells in the pituitary to adjust their output in response to the demands of their target organs. When increased levels of target tissue hormones cause the hypothalamus/pituitary to reduce their stimulation of the target tissue, the process is referred to as *negative* feedback. Negative feedback, which is the most commonly used type of feedback, works to stabilize the operation of the target organ. In some cases, negative feedback occurs directly from the pituitary to the hypothalamus, in the absence of any target tissue response. For example, prolactin is thought to feed back to stimulate the release of dopamine into the portal system which leads to a reduction of prolactin secretion. This type of feedback is known as *short-loop feedback* whereas feedback that involves a target organ is known as *long-loop* feedback.

Steroids represent the most common type of target tissue hormone. Because they are lipophilic they easily cross the blood-brain barrier to act at sites scattered throughout the hypothalamus and basal forebrain. Some neurosecretory neurons synthesize steroid receptors that are used to sense steroid levels directly. Others are not equipped with steroid receptors themselves but depend on “transducer” neurons to supply them with information about circulating steroid levels. The most common way for steroids to affect cellular activity is by binding to intracellular receptors which then bind at specific genomic sites to influence gene transcription. This process is somewhat slow due to the length of time it takes for a change in transcription to alter the cellular concentration of the desired protein. However, under some circumstances steroids have been shown to cause rapid changes in neuronal activity, prompting speculation that membrane steroid receptors also exist in some neurons (39).

Besides their action in the hypothalamus to control the secretion of releasing and inhibiting hormones, steroids also have direct effects on the endocrine cells of the pars distalis. While steroids by themselves do not directly alter pituitary hormone secretion, they modify the response of anterior pituitary cells to hypothalamic hormones. The regulation of gonadotropin secretion by testosterone provides good example of how steroids act at multiple sites to control the release of anterior pituitary hormones. As a negative feedback regulator of LH and FSH secretion, testosterone inhibits pituitary gonadotropin secretion. It does this by acting at the hypothalamus to reduce the frequency (and perhaps the amplitude) of GnRH pulses that are released into the portal circulation (40). In addition, it acts at the pituitary to reduce the response of gonadotropes to the GnRH that is coming from the hypothalamus (41).

## ***Stress***

Stress is a somewhat vague term that covers a variety of psychological and physical stimuli. All pituitary hormones are influenced by stress to some extent. The impact that a type of stress might have on a particular hormone can depend on many different factors including species, age, sex, endocrine status, and time of day. Most stress effects are thought to be mediated by hypothalamic neurosecretory neurons, presumably in response to inputs from higher centers.

## ***Neuroendocrine Interactions***

While it is common to think of each pituitary hormone as being controlled independently by its own hypothalamic neurosecretory system, there is ample opportunity for interaction between the systems at every level from the hypothalamus down to the pituitary, starting with the afferent connections to neurosecretory neurons. As mentioned earlier, input to neurosecretory neurons can consist of both neural and hormonal signals and the same signals can act at sites in more than one neurosecretory system. For example, testosterone, which is the primary negative feedback signal for the control of gonadotropin secretion in the male, not only acts as an input to the GnRH system, but also influences GH release, probably through a direct action on somatostatin neurons (42). In addition, it is common for neurosecretory neurons of one type to establish connections with other types. A good example of this can be found in the CRH system. CRH neurons provide synaptic inputs to GnRH, TRH, and somatostatin neurons while receiving connections from somatostatin and TRH cells.

The anatomical layout of the median eminence also provides a potential substrate for interaction among neurosecretory systems (7). The external zone of the median eminence acts as a terminal site for fibers from CRH, TRH, somatostatin, GHRH, LHRH, and dopamine neurons. Some oxytocin and vasopressin fibers terminate in this region as well. The close proximity of dissimilar terminals and the high concentrations of hypothalamic hormones that are present in the perivascular space makes crosstalk between systems highly probable. Furthermore, numerous neurotransmitter systems innervate the median eminence and many of these are thought to interact with the terminals of more than one type of neurosecretory neurons.

The pituitary represents the final level of interaction between neuroendocrine systems. Hypothalamic hormones have been shown to affect the release of more than one anterior pituitary hormone both *in vitro* and *in vivo*. For example, TRH not only induces TSH secretion, it also stimulates the release of prolactin. Likewise, somatostatin, (named for its growth hormone-inhibiting properties) inhibits TSH and prolactin secretion under some circumstances. The mechanisms responsible for the multiple effects of hypothalamic hormones are not clear. The most obvious possibility is that some anterior pituitary endocrine cells express receptors for more than one type of hypothalamic hormone. Although this is the most commonly accepted explanation, direct evidence that this occurs

in non-tumor cells is meager. Alternatively, anterior pituitary cells could pass chemical messages between each other. This type of communication between cells is called paracrine. In addition to the major hormones that it secretes into the circulation, the pituitary produces a number of bioactive substances that are probably used as paracrine messengers for cell-to-cell communication.

### ***Future Research***

The interactions that can and do occur between the different pituitary hormone regulatory systems serve to highlight the complexities of the neuroendocrine system and make it clear that the work of unraveling the details of how the system operates is far from complete. While traditional neuroendocrine research strategies have provided us with a basic understanding of how pituitary hormones are controlled, continued progress will depend on the use and development of technologies that allow us to look at the system with greater resolution. Among the most promising new tools are those associated with molecular biology. Recently, double label *in situ* hybridization and *in situ* hybridization coupled with immunocytochemical techniques have provided a way to identify subpopulations of cells that express a particular message or protein and measure the expression of another message within those same cells (43). Another approach that has tremendous potential is the development of transgenic animal models. It should be possible for neuroendocrinologists to target the expression of neuroactive peptides and neuropeptide agonists and antagonists to particular populations of hypothalamic neurons or pituitary cells, or to knock out genes that are responsible for the production of specific neuropeptides, enzymes, and receptors. This approach could be used to answer the same kind of questions that are usually addressed in traditional pharmacological experiments but with much greater precision, since only a particular subset of cells would be affected by the manipulation. So, even though the questions that remain to be answered are complex, the utilization of new technologies should insure continued progress in our efforts to understand pituitary hormone regulation.

## FIGURE LEGENDS

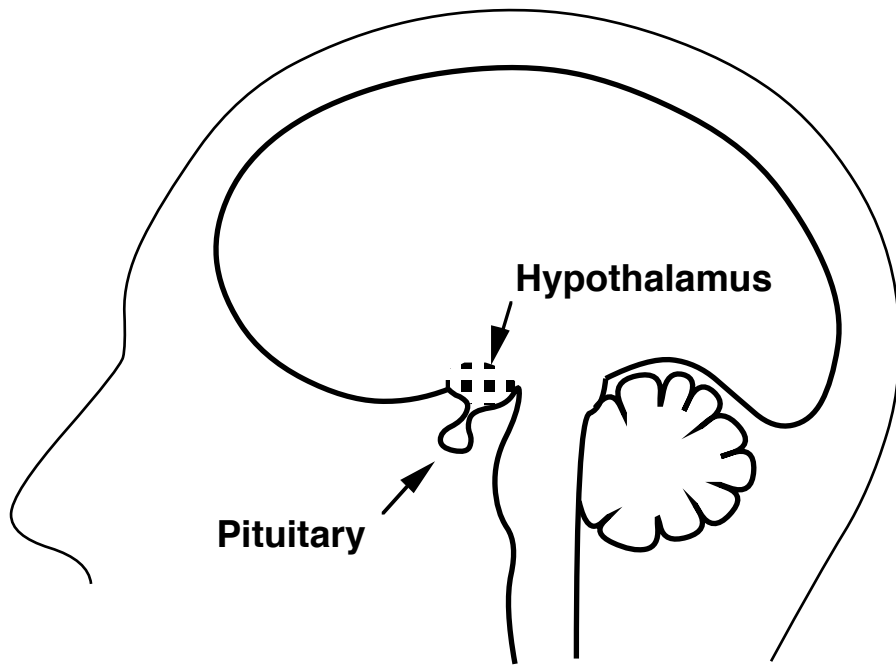
- Figure 1. Relative size and location of the hypothalamus and pituitary in the human.
- Figure 2. Pituitary nomenclature.
- Figure 3. Locations of pituitary hormone-producing cells. The posterior pituitary hormones (oxytocin and vasopressin) are synthesized by neurosecretory neurons in the hypothalamus. Anterior pituitary hormones (LH, FSH, ACTH, TSH, growth hormone and prolactin) are produced by endocrine cells located primarily in the pars distalis of the anterior lobe.
- Figure 4. The pituitary circulatory system. Both the anterior and posterior pituitary contain capillary plexuses into which pituitary hormones are secreted. The capillary bed in the pars distalis (the secondary portal plexus) is connected to another one in the median eminence (the primary portal plexus) through a system of portal veins.
- Figure 5. Relative locations of the major neuroendocrine nuclei of the hypothalamus shown in a sagittal plane.
- Figure 6. Neuroendocrine regulation of the anterior pituitary gland. Hypothalamic hormones are synthesized by neurosecretory cells in the hypothalamus and released into capillary loops of the primary portal plexus located in the median eminence. They are carried down to the secondary portal capillary plexus and act at receptor sites on endocrine cells in the pars tuberalis to control pituitary hormone secretion.
- Figure 7. Some of the neural and endocrine connections involved in the regulation of circadian rhythms in the neuroendocrine system. The primary oscillator, which is located in the suprachiasmatic nucleus, is synchronized to the light cycle by retinal connections through the optic nerve. The suprachiasmatic nucleus influences the activity of hypothalamic neurosecretory neurons and also affects the secretion of melatonin from the pineal gland.
- Figure 8. Long-loop negative feedback. Hormones produced by the target tissue feed back on neurosecretory neurons to reduce their secretion of releasing hormones (or increase their secretion of inhibiting hormones) and on pituitary cells to decrease their sensitivity to releasing hormones.

## REFERENCES

1. **Scharrer B** 1975 Neurosecretion and its role in neuroendocrine regulation. In: Meites J, Donovan BT and McCann SM (ed) *Pioneers in Neuroendocrinology*. Plenum Press, New York, 1:257-265
2. **Wittkowski W, H, Schulze-Bonhage A, H, Bockers T, M** 1992 The pars tuberalis of the hypophysis: a modulator of the pars distalis? *Acta Endocrinol Copenh* 126:285-290
3. **Page RB** 1982 Pituitary blood flow. *American Journal of Physiology* 243:E427-E442
4. **Popa G, Fielding U** 1930 A portal circulation from the pituitary to the hypothalamic region. *Journal of Anatomy (London)* 65:88-91
5. **Bergland RM, Page RB** 1978 Can the pituitary secrete directly to the brain? (Affirmative anatomical evidence). *Endocrinology* 102:1325-1338
6. **Nauta WJH, Haymaker W** 1969 Hypothalamic nuclei and fiber connections. In: Haymaker W, Anderson E and Nauta WJH (ed) *The hypothalamus*. Charles C. Thomas, Springfield, 136-209
7. **Jacobowitz D, M** 1988 Multifactorial control of pituitary hormone secretion: the "wheels" of the brain. *Synapse* 2:186-192
8. **Rodriguez EM** 1972 Comparative and functional morphology of the median eminence. In: Knigge KM, Scott DE and Weindl A (ed) *Median eminence: structure and function*. Karger, Basel, 319-334
9. **Schwanzel-Fukuda M, W. PD** 1989 Origin of luteinizing hormone-releasing hormone neurons. *Nature* 338:161-164
10. **Wray S, Nieburgs A, Elkabes S** 1989 Spatiotemporal cell expression of luteinizing hormone-releasing hormone in the prenatal mouse: evidence for an embryonic origin in the olfactory placode. *Brain Res Dev Brain Res* 46:309-18
11. **Halász B** 1993 Neuroendocrinology in 1992. *Neuroendocrinology* 57:1196-1207
12. **Merchenthaler I, Lopez FJ, Negro-Vilar A** 1990 Colocalization of galanin and luteinizing hormone-releasing hormone in a subset of preoptic hypothalamic neurons: anatomical and functional correlates. *Proceedings of the National Academy of Science* 87:6326-6330
13. **Marks DL, Smith MS, Vrontakis M, Clifton DK, Steiner RA** 1993 Regulation of galanin gene expression in gonadotropin-releasing hormone neurons during the estrous cycle of the rat. *Endocrinology* 132:1836-1844
14. **Vallet PG, Charnay Y, Bouras C** 1990 Distribution and colocalization of delta sleep-inducing peptide and luteinizing hormone-releasing hormone in the aged human brain: an immunohistochemical study. *Journal of Chemical Neuroanatomy* 3:207-214
15. **Meister B, Hokfelt T** 1988 Peptide- and transmitter-containing neurons in the mediobasal hypothalamus and their relation to GABAergic systems: possible roles in control of prolactin and growth hormone secretion. *Synapse* 2:585-605
16. **Delemarre-van de Waal HA, Burton KA, Kabigting EB, Steiner RA, Clifton DK** 1994 Expression and sexual dimorphism of galanin messenger ribonucleic acid in growth hormone-releasing hormone neurons of the rat during development. *Endocrinology* 134:665-671

17. **Niimi M, Takahara J, Sato M, Kawanishi K** 1991 Neurotensin and growth hormone-releasing factor-containing neurons projecting to the median eminence of the rat: a combined retrograde tracing and immunohistochemical study. *Neuroscience letters* 133:183-186
18. **Niimi M, Takahara J, Sato M, Kawanishi K** 1992 Identification of dopamine and growth hormone-releasing factor-containing neurons projecting to the median eminence of the rat by combined retrograde tracing and immunohistochemistry. *Neuroendocrinology* 55:92-96
19. **Shioda S, Nakai Y, Sato A, Sunayama S, Shimoda Y** 1986 Electron-microscopic cytochemistry of the catecholaminergic innervation of TRH neurons in the rat hypothalamus. *Cell and Tissue Research* 245:247-252
20. **Kiss J, Halász B** 1990 Ultrastructural analysis of the innervation of TRH-immunoreactive neuronal elements located in the periventricular subdivision of the paraventricular nucleus of the rat hypothalamus. *Brain Research* 532:107-114
21. **Toni R, Jackson IM, Lechan RM** 1990 Neuropeptide-Y-immunoreactive innervation of thyrotropin-releasing hypothalamic paraventricular nucleus. *Endocrinology* 126:2444-2453
22. **Hisano S, Fukui Y, Chikamori-Aoyama M, Aizawa T, Shibasaki T** 1993 Reciprocal synaptic relations between CRF-immunoreactive- and TRH-immunoreactive neurons in the paraventricular nucleus of the rat hypothalamus. *Brain Research* 620:343-346
23. **Whitnall MH, Mezey E, Gainer H** 1985 Co-localization of corticotropin-releasing factor and vasopressin in median eminence neurosecretory vesicles. *Nature* 317:248-250
24. **Piekut DT, Joseph SA** 1986 Co-existence of CRF and vasopressin immunoreactivity in parvocellular paraventricular neurons of rat hypothalamus. *Peptides* 7:891-898
25. **Pretel S, Piekut DT** 1990 Coexistence of CRF peptide and oxytocin mRNA in the paraventricular nucleus. *Peptides* 11:621-624
26. **Ceccatelli S, Eriksson M, Hokfelt T** 1989 Distribution and coexistence of corticotropin-releasing factor-, neurotensin-, enkephalin-, cholecystokinin-, galanin- and vasoactive intestinal polypeptide/peptide histidine isoleucine-like peptides in the parvocellular part of the paraventricular nucleus. *Neuroendocrinology* 49:309-323
27. **Pretel S, Piekut D** 1990 Coexistence of corticotropin-releasing factor and enkephalin in the paraventricular nucleus of the rat. *Journal of Comparative Neurology* 294:192-201
28. **Abe H, Engler D, Molitch ME, Bollinger-Gruber J, Reichlin S** 1985 Vasoactive intestinal peptide is a physiological mediator of prolactin release in the rat. *Endocrinology* 116:1383-1390
29. **Nikolics K, Mason AJ, Szönyi E, Ramachandran J, Seeburg PH** 1985 A prolactin-inhibiting factor within the precursor for human gonadotropin-releasing hormone. *Nature* 316:511-517
30. **Samson WK, Lumpkin MD, McCann SM** 1986 Evidence for a physiological role for oxytocin in the control of prolactin secretion. *Endocrinology* 119:554-560

31. **Stewart JK, Clifton DK, Koerker DJ, Rogol AD, Jaffe T, Goodner CJ** 1985 Pulsatile release of growth hormone and prolactin from the primate pituitary in vitro. *Endocrinology* 116:1-5
32. **Martínez-de la Escalera G, Choi AL, Weiner RI** 1992 Generation and synchronization of gonadotropin-releasing hormone (GnRH) pulses: intrinsic properties of the GT1-1 GnRH neuronal cell line. *Proceedings of the National Academy of Sciences* 89:1852-1855
33. **Clark RG, Jansson JO, Isaksson O, Robinson IC** 1985 Intravenous growth hormone: growth responses to patterned infusions in hypophysectomized rats. *Journal of Endocrinology* 104:
34. **Belchetz PE, Plant TM, Nakai Y, Keogh EJ, Knobil E** 1978 Hypophysial responses to continuous and intermittent delivery of hypophthalmic gonadotropin-releasing hormone. *Science* 202:631-633
35. **Wildt L, Hausler A, Marshall G, Hutchison JS, Plant TM, Belchetz PE, Knobil E** 1981 Frequency and amplitude of gonadotropin-releasing hormone stimulation and gonadotropin secretion in the rhesus monkey. *Endocrinology* 109:376-385
36. **Schwartz WJ, Gross RA, Morton MT** 1987 The suprachiasmatic nuclei contain a tetrodotoxin-resistant circadian pacemaker. *Proceedings of the National Academy of Science* 84:1694-1698
37. **Hardin PE, Hall JC, Rosbach ME** 1992 Circadian oscillations in period gene mRNA levels are transcriptionally regulated. *Proceedings of the National Academy of Science* 89:11711-11715
38. **Meijer JH, Reitveld WJ** 1989 Neurophysiology of the suprachiasmatic circadian pacemaker in rodents. *Physiological Reviews* 69:671-707
39. **Ramirez VD, Dluzen DE, Ke FC** 1990 Effects of progesterone and its metabolites on neuronal membranes. *Ciba Foundation Symposia* 153:125-141
40. **Steiner RA, Bremner WJ, Clifton DK** 1982 Regulation of luteinizing hormone pulse frequency and amplitude by testosterone in the adult male rat. *Endocrinology* 111:2055-2061
41. **Drouin J, Labrie F** 1976 Selective effect of androgens on LH and FSH release in anterior pituitary cells in culture. *Endocrinology* 98:1528-1534
42. **Burton KA, Steiner RA, Clifton DK** Colocalization of the androgen receptor and somatostatin messenger RNA in neurons in the periventricular nucleus. 20th Annual Meeting of the Society for Neuroscience, St. Louis, 1990 (Abs. 359.11)
43. **Marks DL, Wiemann JN, Burton KA, Lent KL, Clifton DK, Steiner RA** 1992 Simultaneous visualization of two cellular mRNA species by use of a new double *in situ* hybridization method. *Mol Cell Neurosci* 3:395-405



**Figure 1**

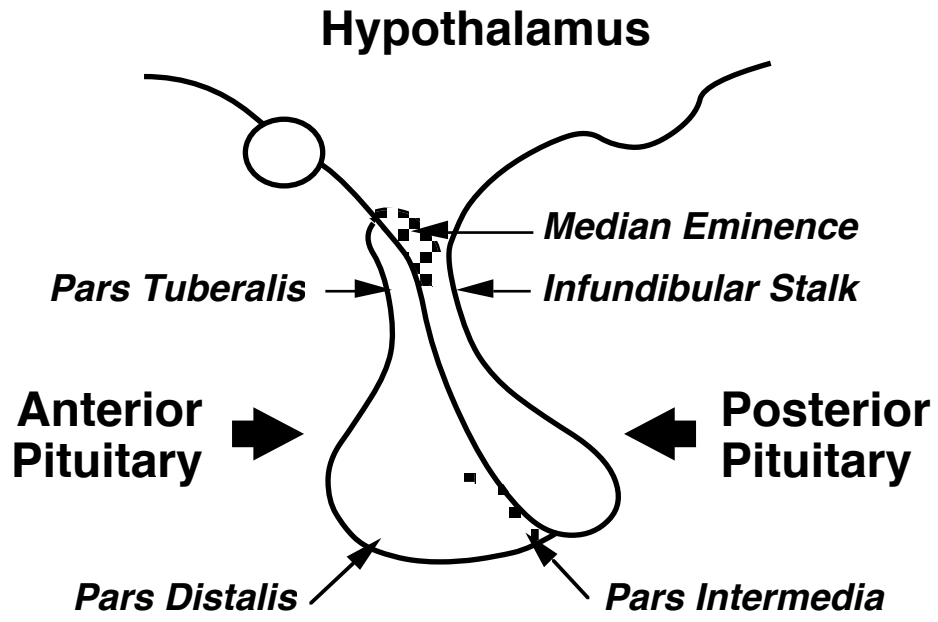


Figure 2

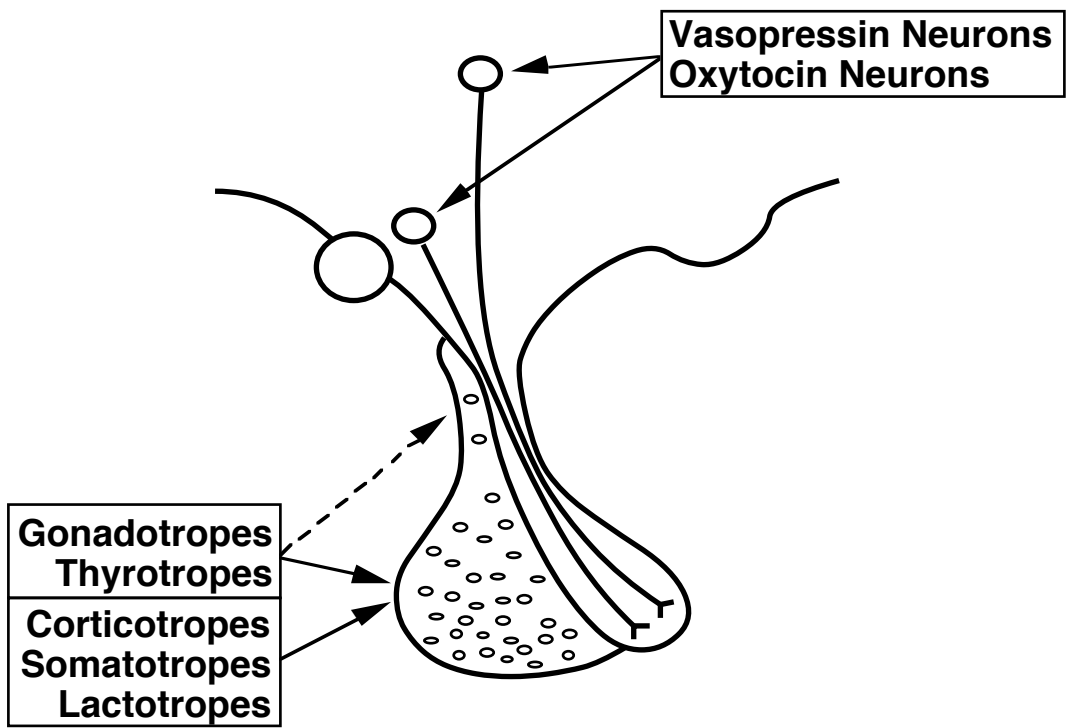
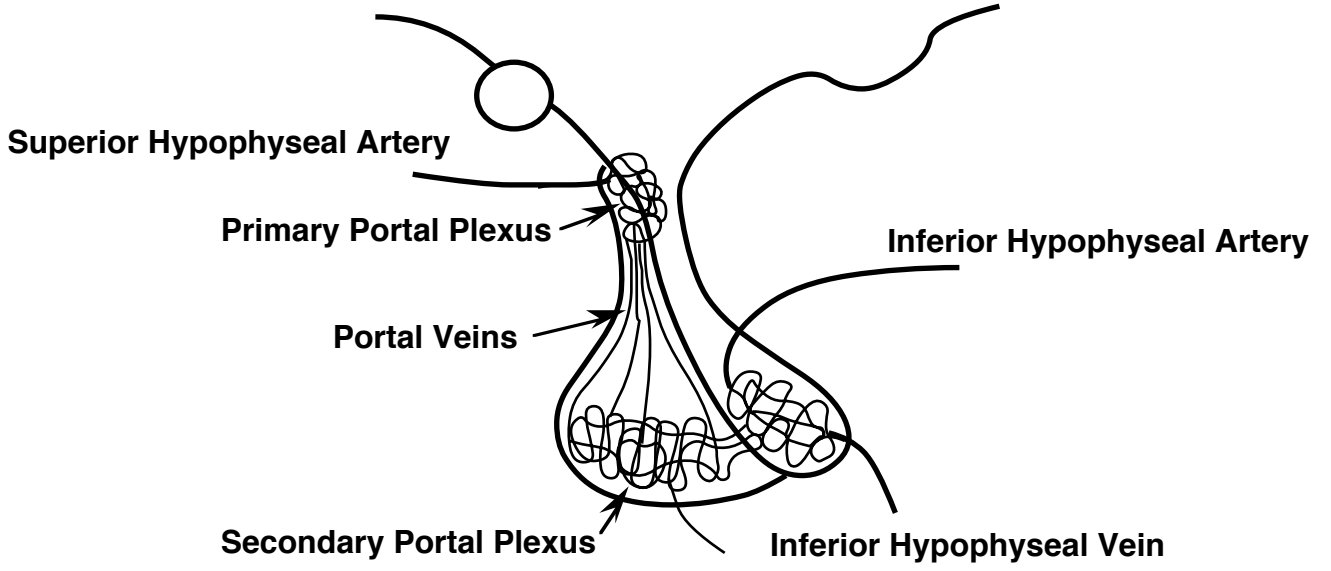


Figure 3



**Figure 4**

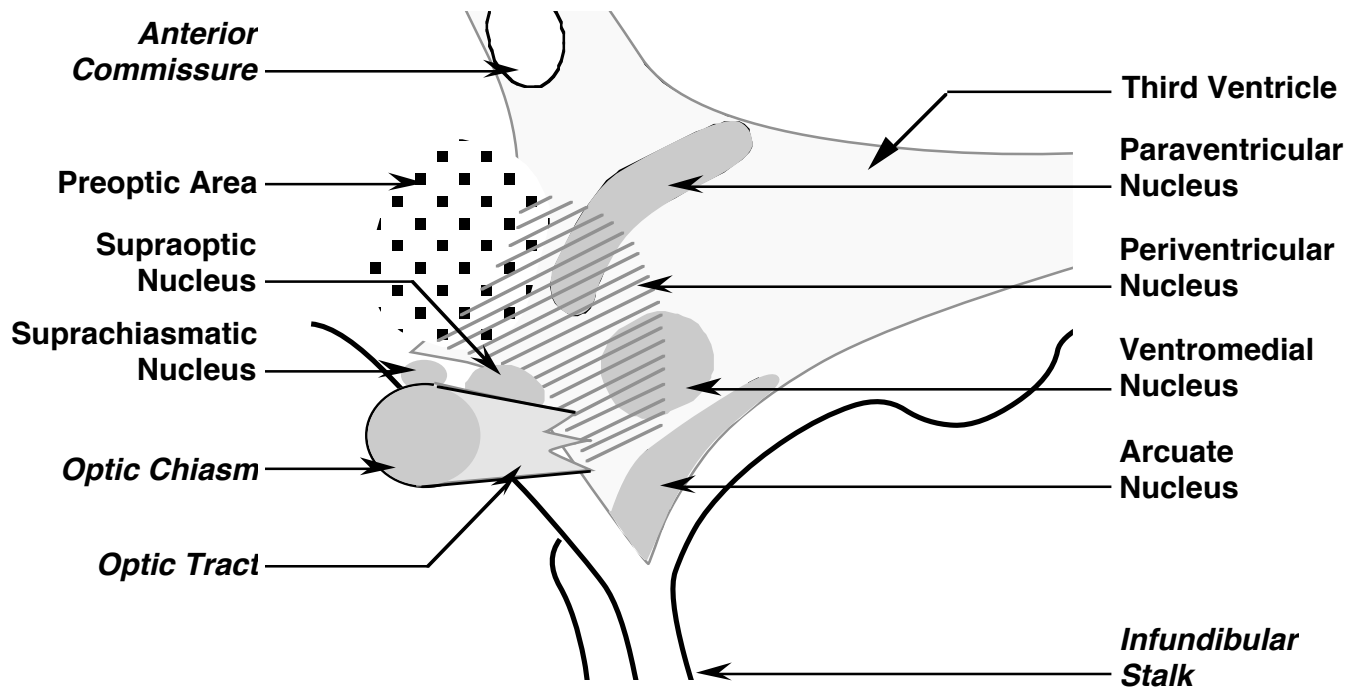


Figure 5

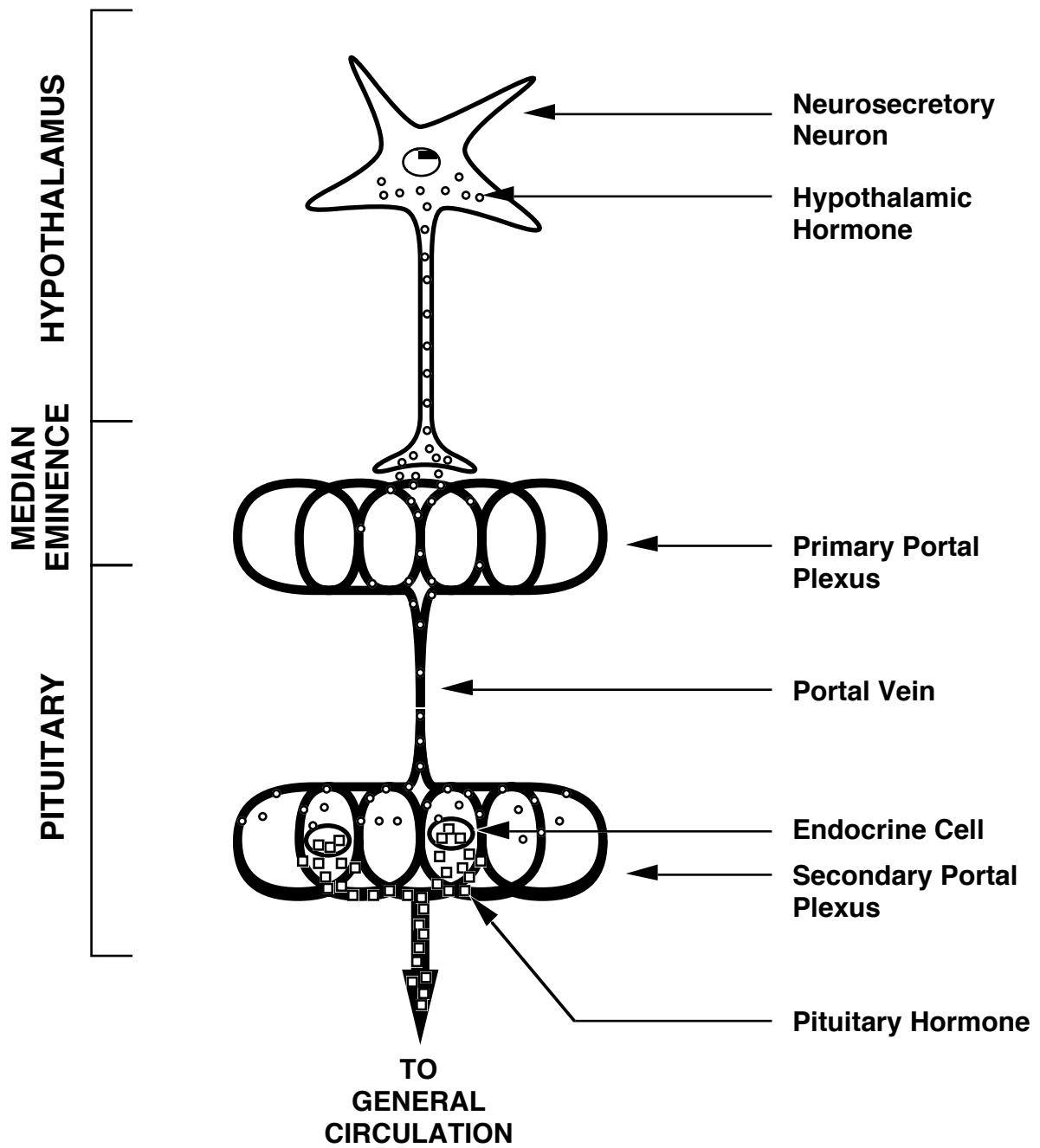


Figure 6

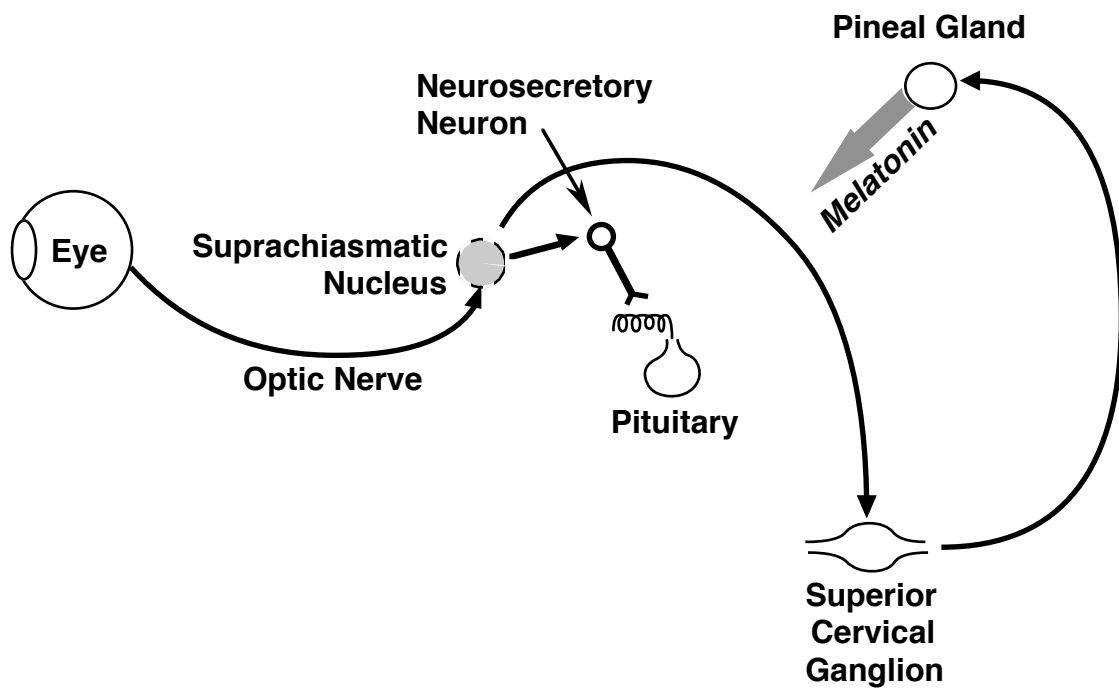


Figure 7

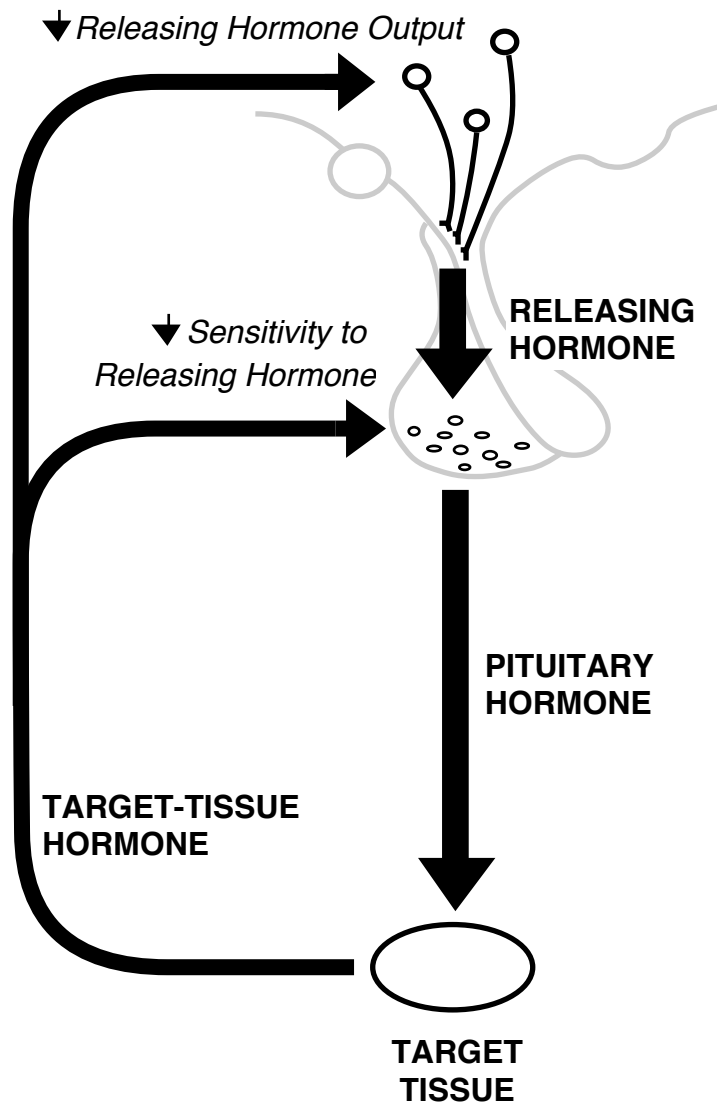


Figure 8