CHAPTER

1

An Introduction to Neuroendocrine Systems

Jon E. Levine

Wisconsin National Primate Research Center, and Department of Neuroscience, University of Wisconsin, Madison, WI, USA

OUTLINE

Neuroendocrinology Defined 3

Neurosecretion 4

The Basic Anatomy of Neuroendocrine Systems 4
- *The Hypothalamus* 4
- *The Pituitary Gland* 6
- *The Hypothalamo-hypophysial Portal Vessel System* 7

Neurosecretory Cells 7

Neuroendocrine Transduction and Neuroendocrine Systems 8

Basic Aspects of Neuroendocrine Integration 9
- *Neuroendocrine Reflexes Mediated by Neurohypophysial Systems* 9
- *The Basic Elements of Homeostatic Neuroendocrine Systems* 10
- *Homeostatic Systems: Hypothalamic—Pituitary—End-Organ Axes* 11
- *Homeostatic Neuroendocrine Systems Controlling Motivated Behaviors* 13
- *Experimental Characterization of Homeostatic Neuroendocrine Systems* 14
- *Environmental Stimuli, Homeostatic Settings and Allostasis* 14

Cellular Mechanisms of Neuroendocrine Integration 15

Clinical Neuroendocrinology 16

The Study of Neuroendocrinology 17

Summary 18

Summary

The field of neuroendocrinology is concerned with the interrelationships between the neural and endocrine systems, and addresses regulatory mechanisms of two basic types: the neural control of hormone secretions, and the actions of peripheral hormones on neurophysiological processes and behavior. In most cases, neuroendocrine systems of both types are linked together in broader bidirectional control mechanisms which govern vitally important processes that include growth, reproduction, metabolism and energy homeostasis, electrolyte and water balance, and responses to stress. The editors and authors of this volume of work have endeavored to provide a comprehensive presentation of the "state-of-the-science" of neuroendocrinology, presented in a format that will be accessible for both generalist and specialist students and researchers who seek a current understanding of neuroendocrine systems. Chapter 1 introduces the basic anatomical and physiological components of neuroendocrine systems, and defines and describes their fundamental operating characteristics.

NEUROENDOCRINOLOGY DEFINED

Neuroendocrinology is a relatively new science that emerged in the mid-20th century as a branch of endocrinology, propelled in part by the realization that the brain produces neurohormones and thereby functions as an endocrine organ. During the same period, fundamental observations were made in the relatively new field of behavioral neuroscience, revealing effects of peripheral hormones on brain function, neural development, and behavior. We now consider that both processes — control of hormone secretions by the brain and effects of hormones on brain function — together define the scope of modern neuroendocrine science. *Neuroendocrine systems* can be defined as the sets of neurons, glands and non-endocrine tissues, and the neurochemicals,

Handbook of Neuroendocrinology, DOI: 10.1016/B978-0-12-375097-6.10001-0

Copyright © 2012 Elsevier Inc. All rights reserved.

Verilux Ex. 1032
Verilux v Litebook
IPR2023-00940
Page 00001

hormones, and humoral signals they produce and receive, that function in an integrated manner to collectively regulate a physiological or behavioral state. *Neuroendocrine integration* is the process by which neuroendocrine systems register, transduce, and interpret important signals from the internal and external environment, and thereafter direct adaptive changes in prevailing physiological and behavioral states. In this introductory chapter, we define and describe different types of neuroendocrine systems, and review the basic integrative mechanisms that each employ to operate under normal physiological circumstances.

NEUROSECRETION

By the 1920s, the existence of the major hormones of the pituitary gland, and their effects on the gonads, adrenal glands and growth, had been established. In the years following, there was greater awareness that the functions of the pituitary gland are largely governed by neural influences. The concept of *neurosecretion* — the production and secretion of neurohormones by neurons, and the actions of these hormones at target tissues — was proposed by Ernst and Berta Scharrer[1] based on their work in fish, and subsequently confirmed in a variety of species in the succeeding decades. This seminal advance is regarded as the launching point for the study of neuroendocrine systems, and perhaps as the start of the field of neuroendocrinology as a whole. Recognition that specialized *neurosecretory cells* can release hormones at neurovascular junctions provided the conceptual framework for understanding the two major neuroendocrine systems that govern pituitary function. In one, neurosecretion of neurohormones (*vasopressin* and *oxytocin*) occurs at neurovascular junctions in the posterior pituitary gland, directly into the systemic circulation to act at distant target tissues. In the other, hypothalamic neurohormones that were later identified as hypothalamic releasing and inhibiting factors,[2,3] are released from neurovascular junctions in the median eminence of the hypothalamus, into a hypothalamo-hypophysial portal vessel system that conveys these factors to their target cells in the anterior pituitary gland. The basic structural and physiological features of these neuroendocrine systems are described below.

THE BASIC ANATOMY OF NEUROENDOCRINE SYSTEMS

The Hypothalamus

In 1859, the celebrated French physiologist Claude Bernard noted that the "constancy of the internal milieu" is essential for the survival and perpetuation of warm-blooded animals. The regulation of the internal state in the face of changing external and internal conditions is *homeostasis* — a process that requires coordinated control over endocrine, behavioral and autonomic nervous system responses to the environment. It is clear that the *hypothalamus* — and the neuroendocrine neuronal systems that reside in it — has evolved to assume a critically important role as a major integrative center for mediating these homeostatic functions. It is located at the base of the forebrain, where it can direct the endocrine functions of the pituitary gland, while also receiving hormonal signals from the periphery. The hypothalamus is also adjacent to — and highly interconnected with — limbic and cortical structures and brainstem autonomic centers. The hypothalamus is uniquely positioned to send and receive endocrine signals, as well as neural signals from sensory systems, emotion- and memory-processing circuitries, and autonomic centers. Neural and endocrine information is continuously transduced, integrated and interpreted in hypothalamic neurons, and appropriate homeostatic signals are conveyed back to these endocrine, autonomic and behavioral control systems to affect coordinated changes, when necessary, in hormone secretions, autonomic outflow and behavioral state. The hypothalamus is thus responsible for monitoring the internal and external environment and coordinating adaptive physiological responses among several systems.

The hypothalamus is defined anatomically as an area of gray matter that is located in the basal forebrain, consisting of two symmetrical halves divided medially by the third ventricle. It emerges in the developing diencephalon to be bounded rostrally by the optic chiasm, caudally by the mammillary bodies, laterally by the optic tracts, and dorsally by the thalamus. The preoptic area (POA) lies rostrally to the hypothalamus, and although it is considered telencephalic in origin, it is often regarded as a functional hypothalamic tissue. Groups of neuronal cell bodies and their neuropils constitute bilaterally symmetrical *hypothalamic nuclei*, as schematically depicted in Fig. 1.1. Although there are species differences in the topography of the major hypothalamic nuclei as well as the less distinct hypothalamic areas, in general the anterior region of the hypothalamus contains the *supraoptic nucleus* (*SON*), *paraventricular nucleus* (*PVN*), *suprachiasmatic nucleus* (*SCN*) and *anterior hypothalamic area* (*AHA*), and a *periventricular nucleus* that continues caudally; the middle region of the hypothalamus includes the *arcuate nucleus* (*AN*), *ventromedial nucleus* (*VMN*), *dorsomedial nucleus* (*DMN*) and *lateral hypothalamic area* (*LHA*); and within the posterior region of the hypothalamus lie the *posterior hypothalamic nucleus* (*PHN*) and the *premammillary nucleus* (*PMN*).

IPR2023-00940 Page 00002

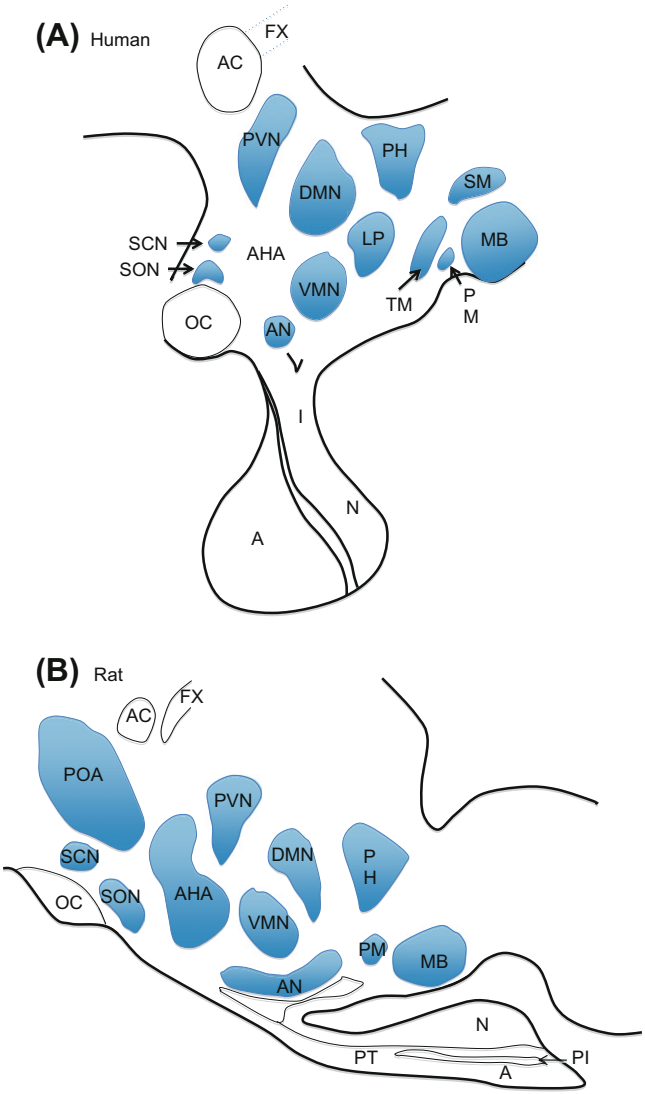

FIGURE 1.1 **Schematic diagrams depicting the anatomical locations of hypothalamic nuclei in parasagittal sections of (A) human and (B) rat basal forebrain.** Figures are oriented as anterior to posterior (rostral to caudal) from left to right. Abbreviations: AC, anterior commissure; A, adenohypophysis; AHA, anterior hypothalamic area; AN, arcuate nucleus; DMN, dorsomedial hypothalamic nucleus; FX, fornix; I, infundibulum; LP, lateral posterior nucleus; MB, mammillary body; N, neurohypophysis; OC, optic chiasm; POA, preoptic area; PH, posterior hypothalamic nucleus; PI, pars intermedia; PM, premammillary nucleus; PT, pars tuberalis; PVN, paraventricular nucleus; SCN, suprachiasmatic nucleus; SM, supramammillary nuceleus; SON, supraoptic nucleus; TM, tuberomammillary nucleus, VMN, ventromedial nucleus.

Classical studies utilized lesion and electrical stimulation techniques to ascribe integrative functions to specific nuclei as "centers" for the control of functions such as feeding, drinking, sexual behavior, stress responses, and electrolyte and water balance. The "center" concept has been somewhat outmoded with the recognition that these regulatory systems are distributed throughout many interconnected neuronal populations within and beyond the hypothalamus. Nevertheless, several of the hypothalamic nuclei do contain well-characterized neurohormone and neurotransmitter-producing cell groups that clearly serve specific integrative and effector functions that are essential components of these systems (Table 1.1). The PVN and SON, for example, are critically important in the regulation of electrolyte and water balance by virtue of the fact that magnocellular neurons within these nuclei produce the antidiuretic neurohypophysial hormone *vasopressin*, as described below; parvocellular neurons within the PVN express *corticotropin-releasing hormone* (CRH) and thereby regulate neural and hormonal responses to stress; the POA similarly contains neurons that produce the central neurohormonal effector of the reproductive axis, *gonadotropin-releasing hormone (GnRH)*, and is therefore essential for the maintenance of gonadal function in rodents; the AN includes neuronal groups that express orexigenic and anorexigenic peptides, as well as receptors for peripheral metabolic hormones, and is thus integrally important in the regulation of food intake and energy expenditure; and subsets of neurons in the VMN express receptors for ovarian hormones, *estrogens* and *progestins*, and mediate many of the actions of these hormones on female sexual behavior. The concept of a neural "center" might be best retained for the case of the SCN, which contains neurons that exhibit circadian pacemaking activity and hence function as a 24-hour clock that controls circadian rhythms in physiology and behavior. The roles played by all of the foregoing cell groups, and the nuclei within which they reside, are described in the context of each of the major neuroendocrine systems characterized in subsequent sections in this and succeeding chapters.

The homeostatic functions of the hypothalamus require that afferent neural signals, derived from brain regions concerned with sensory processing, memory and emotion, are processed and integrated with humoral signals; efferent neural signals that produce appropriate alterations in neurohormone secretions and transmission through efferent pathways are consequently initiated. Not surprisingly, the hypothalamus is known to receive and send neural signals through efferent and afferent pathways that connect it with a ring of subcortical limbic structures known to be critically important in emotional status, such as the *amygdala*; in learning and memory, such as the *hippocampus*; and in autonomic nervous system control, such as the *lower brainstem* nuclei. For example, the major connections between the amygdala and the hypothalamus include the *stria terminalis* and a *direct amygdalohypothalamic* tract; the hippocampus connects with the POA, AN and mammillary bodies via the *fornix*; the *medial forebrain bundle* connects the hypothalamus to the more anterior *septal area* of the basal forebrain; the

IPR2023-00940 Page 00003

TABLE 1.1 Selected List of Chemical and Functional Characteristics of Several Hypothalamic Nuclei in the Rat

Nucleus	Cell bodies producing…	Functional roles
PVN, SON (magnocellular)	oxytocin, vasopressin	Electrolyte and water balance, blood pressure (vasopressin); milk ejection, uterine contractility (oxytocin)
PVN (parvicellular)	CRH, TRH, GR	Stress responses, neurosecretory control of HPA and HPT axis
SCN	vasopressin, VIP	Circadian rhythms
Periventricular nuclei	SST, kisspeptin, ERα, ERβ,	Inhibition of GH secretion, control of ovulatory cyclicity
POA	GnRH, TRH, ERα, ERβ, PR, AR	Neurosecretory regulation of HPG axis, HPT axis; male sexual behavior
AHA		Parasympathetic control, thermoregulation
VMN	GHRH, ERα, PR	Satiety, female sexual behavior
AN	POMC, NPY, AgRP, GHRH, DA, kisspeptin, ERα, PR, GR, leptin receptors	Food intake, energy expenditure, neurosecretory control of PRL and GH
DMN	NPY, GR	Behavioral rhythms, blood pressure, heart rate
PH		Sympathetic control, thermoregulation

Abbreviations not defined elsewhere in this chapter: GR, glucocorticoid receptor; VIP, vasoactive intestinal polypeptide; ERα, ERβ, estrogen receptors of the alpha and beta isoforms; PR, progesterone receptors; AR, androgen receptors; POMC, proopiomelanocortin; NPY, neuropeptide Y; AgRP, agouti-related peptide.

hypothalamus is highly interconnected with the midbrain and lower brainstem nuclei via the *dorsal longitudinal fasciculus*, *mammillotegmental tract* and *mammillopeduncular* tract; and the epithalamus (dorsal posterior segment of the diencephalon that includes the habenula and pineal gland) provides afferents to the POA via the *stria medullaris*.

Within the hypothalamus, well-characterized fiber tracts consist of bundles of axons that extend from the soma of neurosecretory cells, and terminate at sites of neurosecretion. Prominent *paraventricular hypophsyial tracts* and *supraoptic hypophysial tracts* project from the paraventricular and supraoptic nuclei, respectfully, to the posterior lobe. These projections consist of axons of the magnocellular neurons that transport vasopressin and oxytocin to their release sites at neurovascular junctions in the pars nervosa. *Tuberoinfundibular tracts* likewise extend from parvocellular (smaller soma) neurons that produce the hypothalamic releasing and inhibiting factors, to terminate in the median eminence where they release these neurosecretory products into the primary plexus of the hypothalamo-hypophysial portal vasculature.

The median eminence is one of several specialized structures that are located at sites about the cerebroventricular system, and are therefore called *circumventricular organs*. These structures lack the *blood—brain barrier*, in which the endothelium of brain capillaries normally restricts movement of compounds from blood to brain or brain to blood. The endothelia of these organs are typically fenestrated, revealing a morphological basis for the diffusion of substances secreted by neurosecretory neurons into the systemic or portal circulation. Conversely, some of the circumventricular organs clearly serve as targets of circulating factors that may activate neural circuitries. The area postrema, located in the caudal extremity of the fourth ventricle, monitors substances present in the circulation, and serves to trigger emesis as an appropriate response to certain blood-borne stimuli.

The Pituitary Gland

The pituitary gland was at one time considered the master endocrine gland of vertebrates, since it was known to control the activity of other major endocrine glands, such as the thyroid, adrenals and gonads. It is now known to be primarily controlled by hormonal stimuli delivered from the hypothalamus and other glands. The pituitary gland, also known as the *hypophysis*, is comprised of the *adenohypophysis*, alternatively referred to as the *anterior lobe*, and the *neurohypophysis*, also called the *posterior lobe*. The adenohypophysis is primarily glandular tissue, while the neurohypophysis consists of neuronal processes that originate from the soma of neurosecretory neurons in the PVN and SON. These axons pass through the *median eminence*, the mediobasal extremity of the hypothalamus that is continuous with the *infundibulum*, or *pituitary stalk*, and ultimately end in the posterior lobe, or pars nervosa.

The embryologic origins of the two lobes of the pituitary are distinct. The neural lobe arises from the neural

IPR2023-00940 Page 00004

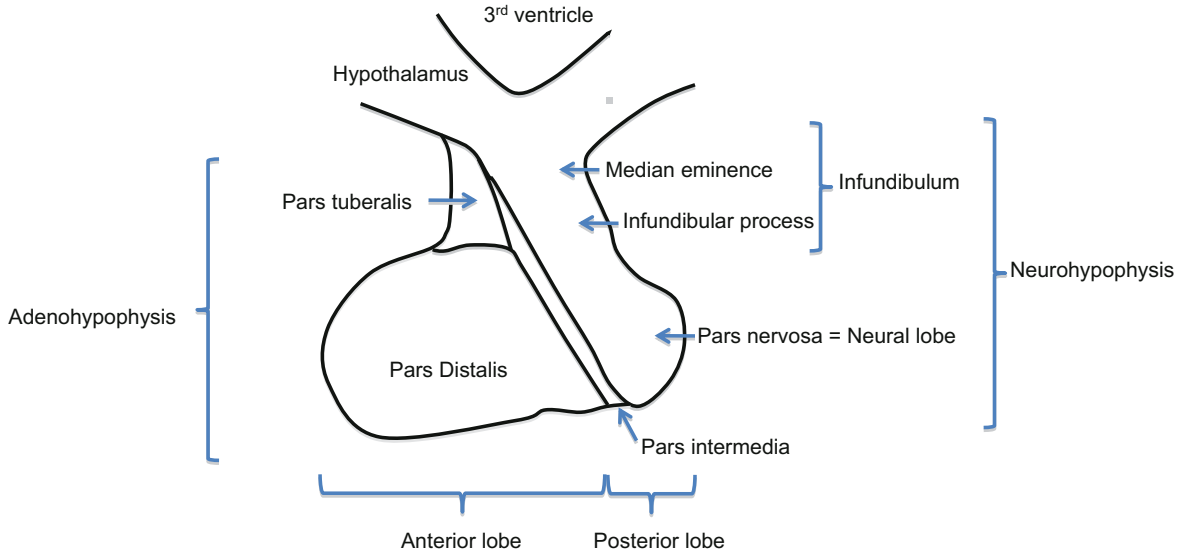

FIGURE 1.2 Anatomical subdivisions of the pituitary gland.

ectoderm of the floor of the developing forebrain, while the anterior lobe is derived from an inward invagination of the primitive mouth cavity (oral ectoderm) known as Rathke's pouch. Cells of the anterior wall of Rathke's pouch develop into the *pars distalis*, containing the majority of the hormone-producing cells of the adenohypophysis. The hormone-secreting cell types and their corresponding hormonal products include the corticotropes (adrenocorticotropic hormone; ACTH), somatotropes (growth hormone; GH), lactotropes (prolactin; PRL), gonadotropes (luteinizing hormone; LH, and follicle-stimulating hormone; FSH) and thyrotropes (thyroid-stimulating hormone; TSH). Dorsal extensions of the anterior lobe constitute a *pars tuberalis*, a non-secretory tissue that wraps around the infundibular stalk. An *intermediate lobe* develops between the two lobes that can vary greatly in size among different species; in humans, this regresses and disappears in adults. In many vertebrates the intermediate lobe produces hormones that include melanotropins, such as *melanocyte-stimulating hormone* (*MSH*). The anatomical components of the pituitary gland are given in Fig. 1.2.

The Hypothalamo-hypophysial Portal Vessel System

The hypophysial vasculature was initially studied in detail in the 1930s, when it was first demonstrated that blood flows downward in the hypophsyial portal vessels, rather than upward from pituitary to brain.[4] Detailed studies thereafter revealed that the superior hypophysial artery provides blood supply to the median eminence and pituitary stalk, from where blood passes via capillary loops through the long portal vessels to the sinusoids of the pars distalis.[5] These findings provided the conceptual basis for Harris' proposal that the hypothalamus exerts a neurohumoral control over anterior pituitary hormone secretions.[6] The pituitary transplantation studies of Harris (described in Chapter 5 of this volume), as well as elegant electrical stimulation experiments by Markee, Sawyer and Hollinshead, as well as Harris,[7,8] confirmed this hypothesis, and in turn revealed the existence of hypothalamic releasing factors that are conveyed by the hypothalamo-hypophysial portal vessel system to control release of anterior pituitary hormones. Two research groups, headed by Roger Guillemin and Andrew Schally, respectively, simultaneously determined the structure of thyrotropin-releasing hormone (TRH), as well as GnRH (or as it was known, LRF or LHRH) and somatostatin (SST), for which these two neuroendocrinologists were awarded the Nobel Prize in Physiology and Medicine in 1977.[2,3] Major releasing factors discovered, in the years following, included corticotropin-releasing hormone (CRH) in 1981,[9] and growth hormone-releasing hormone (GHRH) in 1982 (Fig. 1.3).[10,11]

NEUROSECRETORY CELLS

The major groups of neurosecretory cells in the hypothalamus include those in the neurohypophysial and the tuberoinfundibular systems. Neurons in both groups are predominantly peptidergic, although some may co-produce non-peptide products. An exception is the short-axon tuberoinfundibular dopaminergic (TIDA) neurons that primarily secrete the catecholamine, dopamine, into the portal vasculature. In most regards, neurosecretory neurons are similar in structure and function to neurons elsewhere in the brain. They have

IPR2023-00940 Page 00005

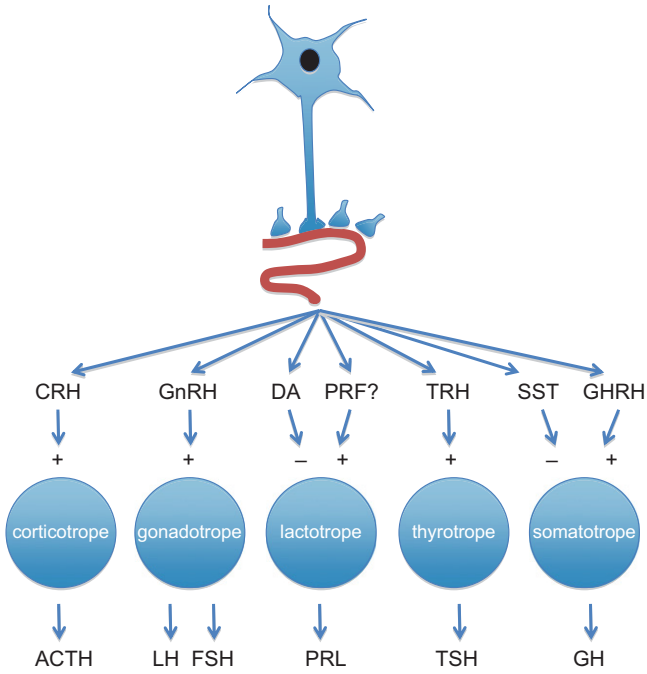

FIGURE 1.3 **Hypothalamic releasing- and inhibiting hormones and their major cellular targets in the anterior pituitary gland.** Abbreviations: ACTH, adrenocorticotropic hormone; CRH, corticotropin-releasing hormone; DA, dopamine; FSH, follicle-stimulating hormone; GH, growth hormone; GHRH, growth hormone-releasing hormone; GnRH, gonadotropin-releasing hormone; LH, luteinizing hormone; PRF, prolactin releasing factor; PRL, prolactin; SST, somatostatin; TRH, thyrotropin-releasing hormone; TSH, thyroid-stimulating hormone.

dendrites, perikarya and axons that resemble those in most central neurons. Furthermore, they exhibit relatively normal resting potentials, action potentials and synaptic potentials, and can display a normal range of intrinsic and synaptically-driven activity patterns that are relatively common among other brain cell types, regardless of their neurotransmitter phenotype. However, the morphology of some neurosecretory neurons, such as the magnocellular vasopressinergic and oxytocinergic neurons, differs in some respects due to the specialized neurosecretory functions. Because they release neurohormones that are delivered via the portal or peripheral circulation to distant target tissues, these neurosecretory cells are required to make copious amounts of neuropeptide hormone in their soma. Thus, these neurons have characteristics of active peptide-producing cells, including abundant rough endoplasmic reticulum, Golgi, and membrane bound granules.

Like other proteins, neuropeptide hormones are synthesized by a ribosomal mechanism. The process of neurohormone gene expression – the transcription of a neurohormone gene and translation of the corresponding mRNA – culminates in the ribosomal synthesis of a pre-prohormone protein that is longer, but inclusive of the specific neurohormone amino acid sequence. It is longer because it contains an amino terminal, hydrophobic signal peptide sequence that functions to permit movement of the protein across the ER membrane into the Golgi apparatus. There, enzymatic removal of the signal sequence, which yields the prohormone, is typically followed by the actions of proteolytic processing enzymes that additionally attack dibasic amino acid cleavage sites. This releases the mature protein hormone sequence from the remaining prohormone precursor molecule, and other peptide sequences with or without biological functions may be produced by these cleavages – for example, the neurophysins in magnocellular neurons. Within the Golgi elements the hormone may additionally be altered by processing enzymes that conjugate carbohydrate, C-terminal amide or other moieties, or create covalent linkages between two sulfur-containing cysteine residues, termed "disulfide bridges." These modifications typically endow the molecule with secondary or tertiary structures that are required for biological activity. Vesicles containing the mature neurohormone are pinched off at the terminal cisternae of the Golgi apparatus, and undergo axonal transport to neurovascular terminals. Release of the neurohormone from the neurosecretory cell thereafter occurs through calcium-dependent *exocytosis*, a process that involves fusion of the secretory vesicle with the plasma membrane and diffusion of its contents into the extracellular space within the neurovascular junction. The neurohormone diffuses through fenestrated capillary walls into the blood.

NEUROENDOCRINE TRANSDUCTION AND NEUROENDOCRINE SYSTEMS

The transformation of neural signals into the release of hormones is called *neuroendocrine transduction*. In general, secretion of neurohormones is a regulated process that reflects neuroendocrine transduction in its simplest form. Both synaptic and hormonal excitation of the neurosecretory cell body initiates action potentials that propagate along the axon and invade the axon terminal. The depolarization of the axon terminal, in turn, triggers an elevation of intracellular Ca^{2+}, which prompts vesicular fusion with the plasma membrane and exocytosis of the granule contents. The amplitude of neurohormone secretion is generally determined by the frequency of action potentials that depolarize the neurosecretory cell terminal. Notably, this stereotyped *stimulus-secretion coupling* process was first characterized by Douglas and Poisner[12] in their classical experiments on isolated posterior pituitary tissues.

Neuroendocrine transduction can take place in the context of several different types of neuroendocrine systems. In the case of the neurophypophysial system,

IPR2023-00940 Page 00006

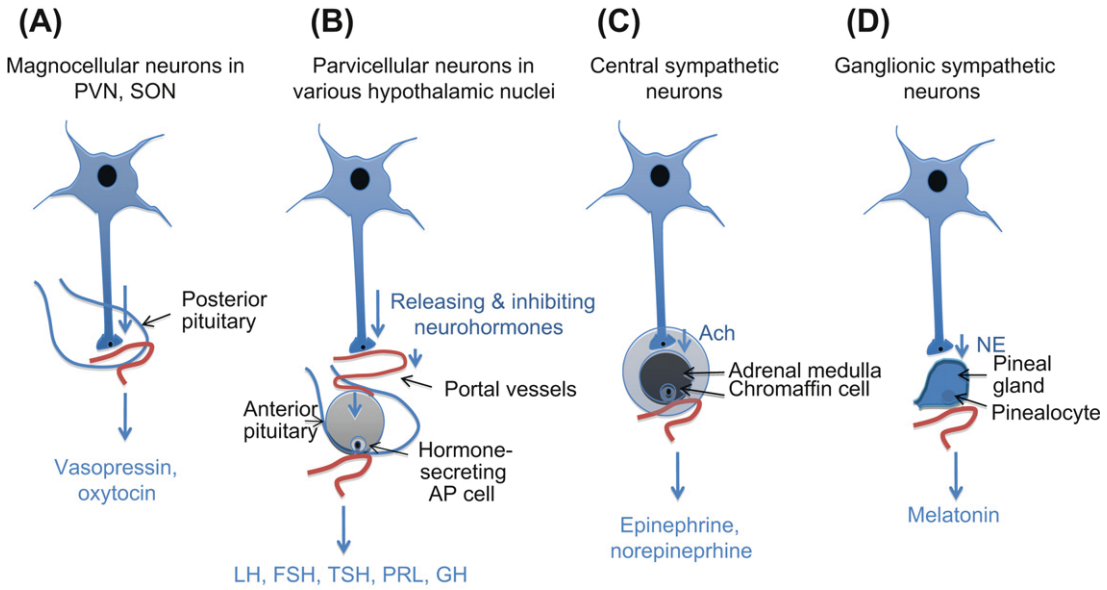

FIGURE 1.4 **Schematic representation of different types of neuroendocrine systems.** (**A**) Neurohypophysial systems releasing vasopressin and oxytocin into the peripheral circulation; (**B**) neurosecretion of hypothalamic releasing and inhibiting hormones into the hypothalamic–hypophysial portal vessel system, regulating anterior pituitary hormone secretions; (**C**) sympathetic innervation of the adrenal medulla, controlling secretion of epinephrine and norepinephrine; (**D**) sympathetic innervation of the pineal gland, controlling secretion of melatonin.

magnocellular neurons in the PVN and SON release vasopressin and oxytocin from neurovascular junctions in the posterior pituitary, into the systemic circulation, through which these hormones are delivered to target organs (Fig. 1.4A). Parvocellular neurons in various hypothalamic nuclei project axons into the median eminence, and release their neuropeptide hormones into the hypothalamo-hypophysial portal vessels, which conduct these neurohormones to the anterior pituitary gland; there, they bind cognate receptors on target pituitary cells and direct the synthesis and secretion of the major trophic hormones of the adenohypophysis (Fig. 1.4B). The sympathetic innervation of the adrenal medulla constitutes yet another variant of a neuroendocrine system; here, axons derived from central sympathetic neurons innervate the chromaffin cells of the adrenal medulla, and at these synapses the neurotransmitter acetylcholine is released, which in turn evokes secretion of epinephrine into the systemic circulation (Figure 1.4C). Similarly, postganglionic sympathetic neurons in the superior cervical ganglion innervate the pineal gland, releasing norepinephrine that in turn evokes release of the hormone melatonin into the bloodstream and cerebrospinal fluid (Figure 1.4D).

BASIC ASPECTS OF NEUROENDOCRINE INTEGRATION

Neuroendocrine regulatory systems can be described as predominantly reflexive or homeostatic control mechanisms. *Neuroendocrine reflexes* resemble their neuromuscular reflex counterparts, as they operate as transient, fixed-pattern or graded reactions to an applied sensory stimulus. The most important and well-studied of these involve the reflexive release of the neurohypophysial hormones, oxytocin and vasopressin. *Homeostatic systems* function to restrict a physiological or behavioral variable such that it is maintained about a set point, or within a restricted range of values. Nearly all neuroendocrine homeostatic systems use some form of *negative feedback* control as a servomechanism that maintains the output of the system, such as secretion of hormones, within a biologically predetermined state. The *hypothalamic–pituitary–end organ* axes, as well as hypothalamic systems that control food intake and other behaviors, are homeostatic systems that feature negative feedback control mechanisms. The basic components of these neuroendocrine systems are described and exemplified below.

Neuroendocrine Reflexes Mediated by Neurohypophysial Systems

Neuroendocrine reflexes mediate acute physiological responses to external and internal signals. A sensory stimulus, such as suckling by an infant at a mother's nipple, can depolarize and thereby activate afferent nerves that convey neural signals up the neuroaxis through multisynaptic pathways to the hypothalamus; these afferent signals activate an effector neuronal population, such as the oxytocinergic magnocellular

IPR2023-00940 Page 00007

neurons, and the net result is a physiological response in the form of neurosecretion and oxytocin-mediated milk ejection that is qualitatively and quantitatively appropriate for the physiological stimulus. Like the classical neuromotor reflexes, neuroendocrine reflexes operate via afferent and efferent loops that can be comprised of mono- or multi-order synaptic pathways. By definition, however, neuromotor reflexes are mediated by neural afferent and efferent pathways. The major neuroendocrine reflexes, by contrast, can be mediated by a humoral input and neural output, humoral input and neural output, humoral input and hormonal output, or neural input and hormonal output. Furthermore, some neuroendocrine reflexes are stereotyped, fixed-action responses to specific stimuli, while others mediate graded responses to stimuli of varying magnitude.

In the foregoing example, the suckling-induced neurosecretion of oxytocin can be considered a fixed-action neuroendocrine reflex mediated by a neural input and hormonal output. One of the major biological actions of oxytocin is to stimulate contractions of myoepithelial cells of the mammary glands, facilitating milk ejection. The reflexive secretion of oxytocin and its actions at the mammary gland represent a fixed-action response, since it is a relatively stereotyped output signal triggered by a specific input signal. The afferent limb of the reflex is comprised of the sensory endings in the mammary glands, primary sensory afferents, and multi-order afferents to the supraoptic and paraventricular nuclei. The suckling stimulus evokes transient increases in the action potentials conveyed along the processes in this afferent limb of the reflex, and thereafter in the firing rate of the oxytocinergic neurons, which is in turn transduced into an acute increase in the neurosecretion of oxytocin from neurovascular junctions in the posterior pituitary gland.[13] The oxytocinergic neurons and the circulation of oxytocin to the mammary gland thus constitute the efferent loop of the neuroendocrine reflex. Suckling-induced oxytocin secretion thus represents a relatively simple reflex circuit that consists of a neural input and hormonal output, and occurs as a fixed-action response to a specific stimulus. A similar neuroendocrine reflex governs the release of oxytocin during parturition, when it assumes an important role in stimulating myometrial contractility. In the latter stages of parturition, dilation of the cervix evokes activity in a neural afferent loop that conveys this mechanoreceptive information to the hypothalamus, culminating in the activation of oxytocinergic neurons and neurosecretion of oxytocin into the systemic circulation. The increase in circulating oxytocin prompts an increase in myometrial activity, and hence in the intensity of the labor. Similar to the milk-ejection reflex, the activation of oxytocin neurosecretion during labor is mediated by a reflex with a neural input and hormonal output, and reflects a fixed-action response to a specific sensory stimulus.

Vasopressinergic neurons also function as effectors of neuroendocrine reflexes. One major biological action of vasopressin is exerted in the distal tubules of the mammalian kidney, where it facilitates water resorption from the collecting ducts. These antidiuretic actions of vasopressin can thereby mediate restoration of blood volume in life-threatening situations such as hemorrhage or severe diarrhea. Vasopressin also induces contraction of vascular smooth muscle cells, stimulating increases in blood pressure. The two major stimuli for the reflexive release of vasopressin are thus decreases in blood pressure and volume, and increases in blood osmolality. Two anatomically distinct afferent loops mediate the transduction of these stimuli into the release of vasopressin. In the first, increased blood osmolality is registered and transduced by osmoreceptive cells in the hypothalamus, and these signals are then conveyed synaptically to magnocellular neurons, prompting activation of these cells and neurosecretion of vasopressin from neurovascular terminals in the posterior pituitary. Increased vasopressin in the circulation thereafter acts via vasopressin receptors in the renal tubules to promote water resorption, and thus dilution of body fluid osmolytes. The distinguishing features of this reflex is that it is comprised of a humoral input and endocrine output, and that it elaborates graded vasopressin secretory responses that are proportional to the magnitude of the initial increase in blood osmolarity. A second reflex involving magnocellular vasopressin neurons is triggered by low blood volume and/or low blood pressure. Baroreceptors located in the aortic arch, carotid sinus and right atrium register any suprathreshold drop in volume or pressure, and tranduce this information into neural signals conveyed up the neuroaxis and ultimately to the magnocellular vasopressinergic neurons; the electrophysiological and thus neurosecretory activity of these cells is accordingly increased, and thereafter the increased vasopressin in the circulation mediates both antidiuretic and pressor responses to the original depressor or hypovolumetric stimulus. The two modes of reflexive vasopressin neurosecretion are mediated by distinct neural input pathways and a common endocrine output (see also Chapter 6).[14]

The Basic Elements of Homeostatic Neuroendocrine Systems

Homeostatic regulation in physiological systems can be described in terms of control systems analysis, using basic terms and concepts borrowed from engineers. A neuroendocrine control system functions to control a physiological variable about a set point, or predetermined range of values, that is most adaptive to the

IPR2023-00940 Page 00008

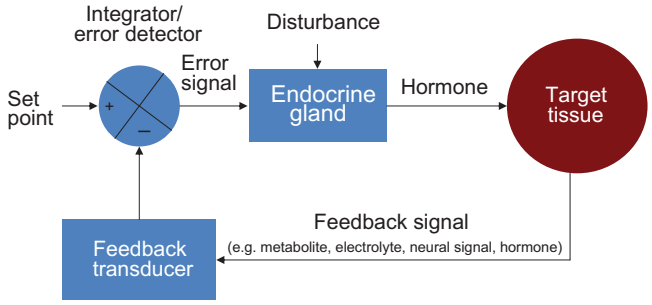

FIGURE 1.5 Basic components of a simple controlled endocrine system featuring negative feedback regulation of secretion (see text for explanation of terms).

animal in a given environment. The generic system features such a set point, an integrator (or error detector, or comparator), a controlling element, a controlled variable, a feedback signal and a feedback signal transducer (Fig. 1.5). This simplest version of a homeostatic control system makes use of negative feedback to maintain the controlled variable within a narrow range of values. The controlling element regulates the controlled variable, which in turn provides a feedback signal representing the momentary value of the variable. A feedback signal transducer registers the feedback signal, converts it to a readable signal, and conveys that information to an integrator. Here, a comparison is made between the ambient level of the variable and a desired set-point value. If a discrepancy is calculated between the real and preselected states, then the integrator delivers an error signal to the controlling elements. In almost all physiological systems, the error signal is inverted in sign to produce an adjustment of the controlling element activity in the opposite direction of the original deviation from the set point. Virtually all neuroendocrine homeostatic mechanisms use some form of negative feedback control that can be described in these terms. Whether there are in fact distinct preselected "set points" that are regulated by simple servomechanisms such as the foregoing control system has been debated, and more complex – and likely distributed – control mechanisms have been proposed to regulate hypothalamic functions, such as thermoregulation.[15] Nevertheless, negative feedback mechanisms, and integrative mechanisms that defend a given range of values along a variable such as body weight,[16] or hormone secretion, are readily demonstrable for most neuroendocrine systems, and thus models such as these prove instructive in understanding the physiological relationships within these systems.

Homeostatic Systems: Hypothalamic–Pituitary–End-Organ Axes

Neuroendocrine homeostatic systems can involve the hypothalamus, anterior pituitary, and an end-organ or target tissues functioning together as an axis. These hypothalamic–pituitary–end-organ axes are organized into distinct tiers of regulated activity, arranged so that hormone signals are conveyed from: (a) hypothalamus to anterior pituitary by hypothalamic releasing and/or inhibiting factors secreted into the portal vessels; and (b) anterior pituitary to end-organ by adenohypophysial hormones secreted into the peripheral circulation. In many of these axes, a third round of signaling proceeds to complete a feedback loop from (c) end-organ back to hypothalamic neurons and/or anterior pituitary gland. In all of these axes, experimental analyses have revealed that negative feedback regulation functions as the predominant control feature within the system. Feedback signals from the anterior pituitary gland to the hypothalamus constitute *short-loop feedback* mechanisms. *Long-loop feedback* mechanisms are those in which feedback signals are conveyed from the end-organ, such as the gonad, thyroid or adrenal cortex, to antecedent levels.

The three-tiered neuroendocrine systems include: (a) the hypothalamic–pituitary–thyroid (HPT) axis; (b) the hypothalamic–pituitary–gonadal (HPG) axis; and (c) the hypothalamic–pituitary–adrenocortical (HPA) axis (shown schematically in Figs 1.6A, B and C, respectively). In the HPT axis, a population of hypothalamic neurons primarily located in the PVN produces the releasing factor TRH, and secretes the tripeptide from neurovascular terminals in the median eminence. The TRH peptide is conveyed to the anterior pituitary gland in the portal vessels, where it diffuses through the fenestrated secondary capillary plexus into the interstitial spaces of the anterior pituitary gland; here, the peptide can bind to G-protein coupled TRH receptors on thyrotropes and cytoplasmic signaling cascades that in turn stimulate synthesis and secretion of thyrotropin (or thyroid-stimulating hormone, TSH). Thyrotropin is conveyed via the circulation to the thyroid, where it binds TSH receptors on follicular cells and promotes the production of T3 and thyroxine (T4). Thyroid hormones exert their widespread actions on target tissues via their cognate intracellular receptors, while also providing the major negative feedback signal within the axis. Thus, elevations in T3 and T4 exert long-loop feedback actions at the hypothalamic level to suppress TRH expression and neurosecretion, and at the pituitary level to suppress TRH-stimulated TSH secretion. Conversely, reduction in thyroid hormone, or complete removal thyroid hormones by thyroidectomy, is generally accompanied by elevations in TRH and TSH secretions.

Similar cascades of hormonal activity and feed back relationships prevail in other hypothalamic–pituitary–end-organ axes. As described fully in succeeding chapters in this book, the HPG axis and the HPA axis

IPR2023-00940 Page 00009

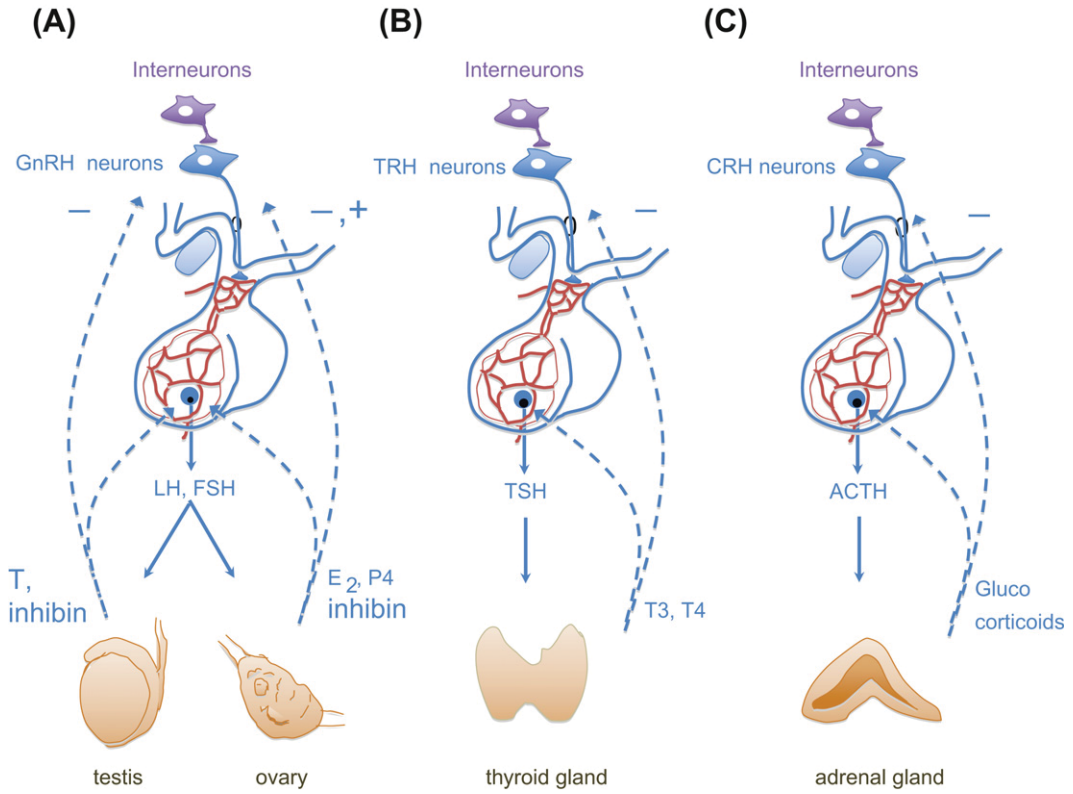

FIGURE 1.6 **"Three-tiered" (A–C) and "two-tiered" (D, E) homeostatic neuroendocrine systems.** (**A**) Hypothalamic–pituitary–gonadal (HPB) axis; (**B**) hypothalamic–pituitary–thyroidal (HPT) axis; (**C**) hypothalamic–pituitary–adrenocortical (HPA) axis; (**D**) hypothalamic control of GH secretion; (**E**) hypothalamic control of PRL secretion. Shown for each axis in (A–C) are the major hypothalamic releasing neurohormones, and their corresponding pituitary and end-organ hormone mediators. Homeostatic control in each axis is largely effected by long-loop negative feedback mechanisms, mediated by end-organ action in the hypothalamus and/or anterior pituitary gland. Hypothalamic feedback can be mediated by end-organ hormone actions on interneurons controlling the releasing factor neurons, or on the releasing factor neurons themselves. Feedback in the hypothalamic–pituitary–gonadal axis is exerted by gonadal steroids (primarily testosterone (T) in males, and estradiol (E_2) and progesterone (P4) in females), as well as the protein hormone inhibin, which selectively suppresses FSH secretion in both sexes. Triodothyronine (T3) and thyroxine (T4) exert feedback in the HPT axis, and glucocorticoids, principally cortisol in humans and corticosterone in rodents, exert major feedback effects in the HPA axis. Shown for the two-tiered systems in (D) and (E) are their hypothalamic releasing and inhibiting hormones, corresponding pituitary hormones, and major target tissues. Homeostatic controls in each of these systems are largely affected by short-loop feedback mechanisms mediated by GH and PRL actions in hypothalamic neurons controlling releasing of their corresponding releasing and inhibiting factors.

also function as three-tiered hormonal control systems in which long-loop feedback mechanisms predominate. Like the HPT axis, the HPG and HPA axes are organized so that the primary releasing factors (GnRH and CRH) stimulate their corresponding trophic pituitary hormones (gonadotropins and ACTH), which in turn stimulate end-organ hormones (gonadal steroid/peptide hormones, and glucocorticoids), which in turn exert long-loop feedback effects at preceding levels in the respective axis.

Secretions of GH and PRL are predominantly under the control of two-tier systems in which short-loop feedback functions are the major regulatory mechanism. The reduced importance of long-loop feedback control in these systems is probably a function of the distributed targets of GH and PRL actions. Growth hormone exerts actions in bone, cartilage, liver, muscle and other tissues, while PRL evokes responses in mammary tissue, gonads and accessory sex organs. Without a single endocrine end-organ to provide feedback control, GH and PRL have evolved the capacity to exert their own direct feedback control within the hypothalamus, ultimately influencing release of the hypothalamic releasing and inhibiting factors that control their own secretions. Furthermore, both stimulatory and inhibitory hypothalamic mechanisms have evolved to control GH and PRL secretion. Hypothalamic neurosecretion of GHRH stimulates, while SST inhibits, GH secretion; and dopamine inhibits, while one or more putative PRL-releasing factors stimulate, PRL release. The short-loop feedback mechanisms for both hormones appear to be mediated by both suppression of releasing factor (GHRH, PRL-RFs) release and stimulation of inhibitory factor (SST, dopamine) release. In the case of GH control, it should

IPR2023-00940 Page 00010

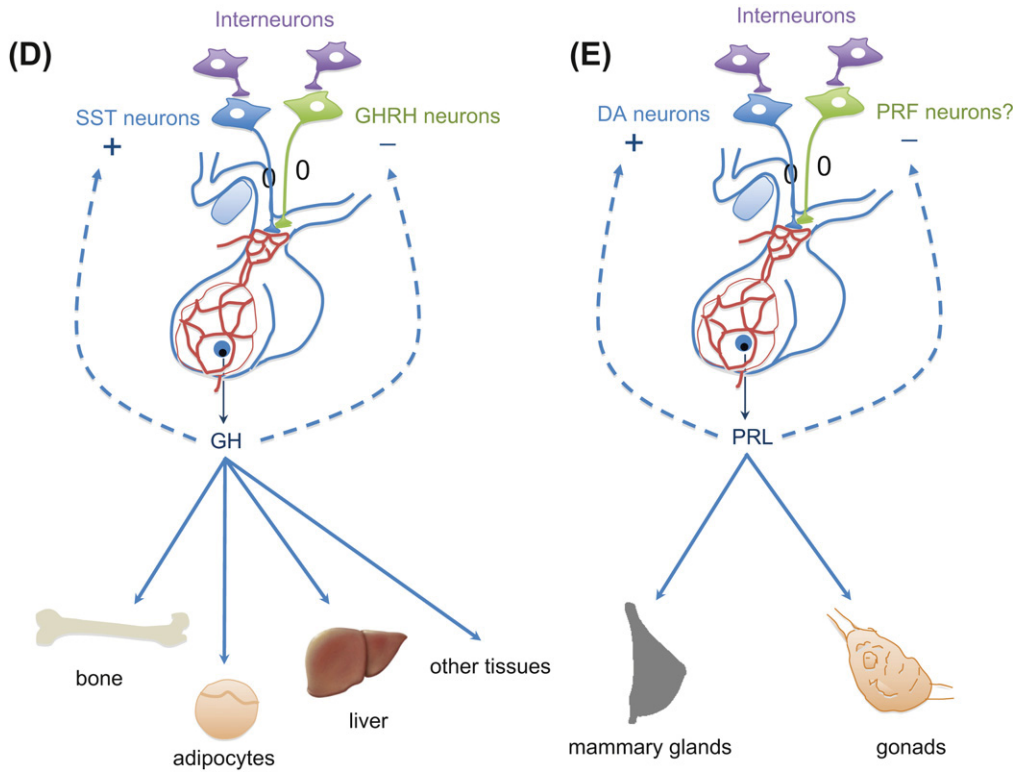

FIGURE 1.6—(*Continued*).

be noted that some long-loop feedback regulation has been shown to be superimposed upon the basic short-loop feedback control system — the actions of GH in promoting cartilage formation, leading to bone growth, are mediated in part by GH stimulation of somatomedins (insulin-like growth factors 1 and 2; IGF1, IGF2) from liver, and IGF1 may also exert negative feedback actions within the hypothalamus to suppress GHRH and stimulate SST release.

Homeostatic Neuroendocrine Systems Controlling Motivated Behaviors

Specific hypothalamic neuronal populations and circuitries have been shown to be intimately involved in the regulation of motivated behaviors, and to function as major targets of peripheral hormones and other humoral factors that regulate these behaviors. Many of the chapters in this book address in detail the neuroendocrine mechanisms that mediate the central control of motivational states that prompt reproductive, social and ingestive behaviors. Some of these mechanisms function as homeostatic neuroendocrine systems that feature stimulatory and inhibitory influences and both short-term and long-term feedback controls. This is perhaps best exemplified by the neuroendocrine systems that govern energy homeostasis.

Food intake and energy expenditure — and hence body weight — are controlled within narrow limits by homeostatic neuroendocrine systems. While these homeostatic control mechanisms have been known to exist for many decades, neuroendocrine research has only recently revealed several of their major endocrine and neurochemical components. Neurons located in the mediobasal hypothalamus are now known to be critically important in both food intake and the autonomic and behavioral systems through which energy expenditure is controlled. Many of these hypothalamic neurons produce neuropeptide neurotransmitters that are either orexigenic (stimulate food intake) or anorexigenic (inhibit food intake), while at the same time reduce or increase energy expenditure. The relative activities of these cell groups are believed to manifest the prevailing motivational state (i.e., satiety or hunger). These cell groups are influenced, in turn, by neural and hormonal signals that convey information about the availability of metabolic energy substrates. Some of these signals arise from the gut and provide short-term cues about the absence or presence of food in the stomach.[16,17] Others are hormonal signals that represent the levels of metabolic substrates in blood, and stored in fat cells and other tissues. The adipocyte-derived hormone, leptin, is one such hormonal modulator that has been shown to suppress food intake and increase energy expenditure.[18]

IPR2023-00940 Page 00011

Insulin appears to exert similar feedback effects.[19] The targets of leptin and insulin feedback effects on food intake and energy expenditure are the same hypothalamic neuropeptidergic neurons that are crucial in inducing or inhibiting feeding behavior and energy expenditure. The long-term homeostatic regulation of body weight thus appears to be controlled by a neuroendocrine mechanism that controls energy intake and expenditure, and a negative feedback regulation of these same neuronal groups by leptin, insulin and other factors that are raised as a consequence of energy intake and storage. How the hypothetical set point for bodyweight regulation may be established, and how it may be altered in morbidly obese individuals, remains the subject of intense study.

Experimental Characterization of Homeostatic Neuroendocrine Systems

Homeostatic neuroendocrine systems have been characterized through controlled experimental manipulation of the neuroendocrine axis, followed by careful analysis of the responses of the system. Selective blockade or stimulation of forward signals in the axis, or removal and replacement of feedback signals, produces responses of the system that are predicted by a control system theory. For example, in the HPG axis, the stimulatory actions of GnRH on LH secretion and LH on testosterone secretion are easily demonstrated *in vivo* and *in vitro*. Conversely, removal of these regulators *in vivo*, by immunoneutralization, pharmacological methods, hypothalamic lesions or hypophysectomy, results in a precipitous decline in circulating testosterone concentrations. Negative feedback control has also been characterized in virtually all of the neuroendocrine axes. The critical test requires removal of the end-organ, where possible, and measurement of the hypothalamic and pituitary secretory responses. In systems featuring negative feedback, removal of the end-organ (e.g., gonads) results in an acceleration of GnRH release and a large increase in LH secretion; similarly, removal of the adrenal glands results in a robust increase in CRH and ACTH secretion, and removal of the thyroid results in a substantial increase in TRH and thyrotropin secretion. In all cases, replacement with physiological concentrations of the appropriate end-organ hormone prevents these increases in hypothalamic and pituitary hormone secretions. Specific features of these control mechanisms can differ between the axes. For example, in some the principal feedback target may be the hypothalamus, and in others the pituitary may serve as the primary feedback target. Furthermore, in the systems governing GH and PRL secretion, short-loop feedback control may involve both suppression of the releasing factor and stimulation of the inhibiting factor.

Environmental Stimuli, Homeostatic Settings and Allostasis

While defending a preselected state, neuroendocrine homeostatic systems must also register and transduce acute stimuli, mount an appropriate response to the perturbation, and return the system to the basal operating state. In the HPA axis, for example, a stressful stimulus rapidly evokes neurosecretion of CRH into the portal vessels, raising ACTH and hence glucocorticoid release. The duration and pattern of the CRH, ACTH and glucocorticoid response will be limited to some extent by an increase in feedback suppression of CRH and ACTH; however, with unabated stress some elevated level of CRH and ACTH secretion would persist. Termination of the stress is then accompanied by a return to the original levels of CRH, ACTH and glucocorticoid secretions, and responsiveness to feedback in the system.

There are numerous examples of short-term responses by an axis to a physiological stimulus. These perturbations usually take the form of exteroceptive or interoceptive stimuli that are conveyed by sensory pathways, and they can be pheromonal, thermal, visual, auditory, tactile, olfactory or gustatory in nature. In reflex ovulators, for example, coitus stimulates neural pathways of virtually all of the sensory modalities, and these sensory signals converge in the hypothalamus. Here, integrative neurons produce a major ovulatory release of GnRH into the portal vessels, triggering a surge of LH into the circulation that in turn evokes ovulation.[20] This neuroendocrine mechanism ensures that the ovulation occurs in temporal register with the presence of viable sperm in the reproductive tract, increasing the chances of reproductive success. Following the perturbation of the HPG axis, it returns to its basal activity state under the control of steroid hormone feedback mechanisms. Another example is the response of the HPT axis to an acute cold stress, which produces a transient stimulation of TRH, and hence TSH and thyroid hormone secretions[21]; the latter secretions serve to elevate body metabolism and mount a thermoregulatory response to the external cold stress. Interoceptive sensory signals can likewise be conveyed from baroreceptors and proprioceptors in peripheral organs and tissues to the hypothalamus, and thereby effect changes in neurohormone output. A sudden drop in blood pressure, for example, is detected by baroreceptors in the atria, carotid bodies and elsewhere in the arterial system, and resulting neural signals are conveyed up the neuroaxis, into the hypothalamic centers that mediate stress responses. As a result, CRH and thus ACTH and glucocorticoid secretions (as well as vasopressin release) are acutely increased.[22] Restoration of blood pressure is accompanied by a return of the

IPR2023-00940 Page 00012

HPA axis to its original basal activity state. Interoceptive signals can also be mediated by alterations in circulating concentrations of metabolic intermediates, osmolytes or growth factors. Specific neuronal populations function as sensory receptors, which monitor the blood levels of these physiological factors and relay appropriate commands to hypothalamic homeostatic regulatory systems. For example, elevated amino acid levels (e.g., arginine) in blood stimulate GHRH and GH secretion, while hypoglycemia induces CRH/ACTH/glucocorticoid secretion.

Homeostatic systems do not function in isolation from one another. Often a most adaptive state may entail responses that are integrated among the different homeostatic neuroendocrine systems. In some cases, these responses are synergistic; stressful stimuli activate both CRH and vasopressinergic neurosecretion in the median eminence (distinct from the magnocellular vasopressin release from posterior pituitary), and vasopressin can amplify the effects of CRH on ACTH secretion from corticotropes.[23] In other circumstances, the activation of one system is accompanied by the inhibition of another, providing an overall net adaptive benefit for the animal. Stressors of many types, as well as a prolonged state of negative energy balance that accompanies undernutrition or strenuous exercise training, raise stress hormone secretions while often suppressing reproductive hormone secretions.[24] These coordinated responses to environmental conditions and behavioral state are mediated in large part by activation of CRH neurons and suppression of GnRH neurons, through mechanisms that are largely unknown and are the subject of current studies. Clearly, the induction of "hypothalamic amenorrhea" in these circumstances is an adaptive response that conserves physiological resources that increase the chance of individual survival, at the expense of temporary suppression of reproductive activities that normally perpetuate the species.

As revealed by Bernard, homeostatic systems maintain the constancy of the internal milieu in the face of changing external conditions. However, homeostatic systems also confer a major adaptive advantage to the organism, because homeostatic settings can change during development, and can be altered in anticipation, in association and/or in response to changes in the internal and external environments. An example of the resetting of a homeostatic set point during development is the increase in the frequency and/or amplitude of GnRH secretion that occurs during the pubertal activation of the HPG axis.[25,26] The pubertal activation of GnRH release is sustained throughout the adult reproductive lifespan, as is the acquired equilibrium between hormone actions and feedback mechanisms in the HPG axis. The set point for neurohormone secretion can also vary sinusoidally over the circadian period, likely as a function of signals from the biological clock in the SCN; activity in the HPA axis, for example, varies with a 24-hour rhythm that peaks in the morning hours and decreases to a nadir in the evening. Sustained shifts in the set point of an axis can also occur over many days; pheromonal cues, for example, can manifest continued inhibition of GnRH neurosecretion and hence reproductive state, while social and psychological cues can alter food intake and energy homeostasis in humans. Seasonal alteration in reproductive status (seasonal breeding) is a reproductive strategy that represents major set-point adjustments in the HPG axis over periods of months. The reproductive axis receives photoperiodic cues through a signaling cascade that includes a retinohypothalamic pathway, synaptic pathways leading to the pineal gland, nocturnal melatonin secretions and, ultimately, neuronal circuitries that control GnRH neurosecretion. Information regarding day length is registered, transduced, and encoded in the duration of melatonin secretion as stimulatory or inhibitory signals for reproductive status. The resulting periods of reproductive activity and inactivity are thereby sustained throughout the months that are most adaptive for the reproductive success and survival of young of a particular species.

Some of the foregoing adaptive changes in set points can be considered as components of higher-level integrative and adaptive responses to changing environments and social, ecological, and physical variables. Superimposed upon the concept of homeostasis is the idea of *allostasis*, or achieving stability through change. Allostasis is thought of as a process that supports and maintains homeostatic systems to allow the organism to adapt to changes in the environment and life history stages.[27] Major mediators of allostasis include the HPA axis, catecholamines and cytokines, and allostatic state refers to altered and sustained activity of these mediators in response to environmental challenges. The adaptation-promoting responses of these mediators are recognized as having protective action in the short term, but may cause damage over the long term, including increased rates of brain and body aging, poor responses to stress, and increased susceptibility to metabolic, cardiovascular and psychiatric disease, as well as immune compromise. *Allostatic load* refers to the accumulated wear and tear caused by inefficiently operated adaptive responses over time.

CELLULAR MECHANISMS OF NEUROENDOCRINE INTEGRATION

Both neuroendocrine reflexes and homeostatic regulatory systems are critically dependent upon the functioning of the cellular signal transduction mechanisms

IPR2023-00940 Page 00013

in their constituent parts, especially those that occur within the hypothalamic neurons that control these systems. A given hypothalamic cell is endowed with certain complements of neurotransmitter and hormone receptors, second messenger systems, transcription factors and ion channels, all of which may determine the hypothalamic cell's role in receiving, integrating and transmitting neural signals that are vital to the operation of a given neuroendocrine system. A major challenge to neuroendocrine researchers is to elucidate mechanisms through which a cell registers neural and hormonal signals and integrates them in a process leading to the production of a physiological output signal. The difficulty of this task is greatly compounded by the tremendous complexity of cell–cell connections and interactions with the hypothalamus, and the likelihood that individual neurons are responsible for integrating many different types of signals conveyed simultaneously, or in an ordered temporal sequence. Signals to be integrated include: (a) synaptic activation via afferent neural pathways; (b) circulating hormones that bind their cognate receptors in the cell, thereby altering cytoplasmic signaling cascades and hence electrophysiological activity, secretory activity, and/or the expression of a variety of target genes; and (c) other humoral factors, such as metabolic intermediates, electrolytes, temperature, etc. Moreover, humoral or neurochemical actions may be stimulatory or inhibitory, or they may be permissive – that is, they render a cell more or less responsive to another (or the same) stimulus. Cellular integration occurs when a cell assesses the weight of each of these signals, computes their net effect and directs alterations in output signals. The final output signal is encoded in changes in the rate or pattern of neurotransmission or neurosecretion from that cell.

A fundamental property that is specific to many neuroendocrine cell groups is the propensity to release neurohormone in synchrony and at regular intervals. This coordinated, intermittent release pattern by a population of neurosecretory cells is referred to as *neuroendocrine pulsatility.* An example of pulsatile GnRH release from terminals in the median eminance and the corresponding pattern of pulsatile LH secretion in peripheral blood of an ovariectomized ewe is shown in Fig. 1.7.[28] Classic experiments by Ernst Knobil and colleagues[29] established the functional importance of pulsatile neurohormone secretion, as presentation of continuous neurohormonal stimuli is less effective, or even inhibitory, in releasing pituitary hormones. However, administration of neurohormone (e.g., GnRH) as a series of regular pulses can continue to evoke anterior pituitary hormone secretions for virtually unlimited periods. It appears likely that pulsatile hormone secretions serve to maintain responsiveness of the signal transduction events in pituitary cells, instead of inducing downregulation

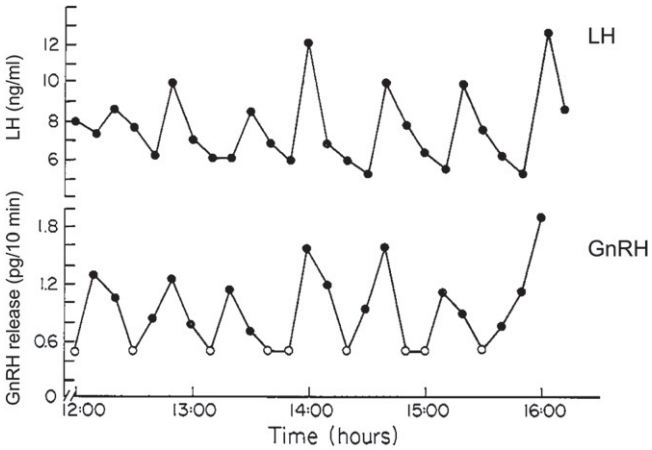

FIGURE 1.7 Pulsatile hypothalamic GnRH release and LH secretion in serum as simultaneously monitored in an ovariectomized ewe. *Modified from, Levine* et al.[28]

of receptors or their downstream signaling pathways. The pulsatile pattern of neurohormone secretion has been found to be of profound importance in clinical cases requiring neurohormone substitution therapies, such as the GnRH regimens used to induce or restore fertility in hypogonadotropic-hypogonadal patients.[30]

Neural and hormonal signals may also exert long-lasting, often permanent, effects on the function of a hypothalamic cell group by altering neurogenesis, cell growth, axon and dendrite morphogenesis, synaptic connectivity, and cell death during fetal and neonatal development, as well as neuroplasticity in adulthood. The well-known organizational effects of steroid hormones during fetal development are but one class of trophic signals that impact hypothalamic function. Metabolic hormones, such as leptin, have also been determined to exert trophic effects on developing hypothalamic circuitries.[31] Many of these developmental actions of hormones are discussed in subsequent chapters, particularly with relevance to sexual differentiation of the brain or prenatal or neonatal programming of neuroendocrine function and behavior.

CLINICAL NEUROENDOCRINOLOGY

Clinical consequences of neuroendocrine pathophysiologies are discussed in many of the chapters in this book, although in general they fall into categories of growth and developmental impairments, infertility, oligo- or galactorrhea, hypo- or hyperthyroidism, hypo- or hyperadrenocorticolism, diabetes insipidus, feeding disorders, metabolic diseases, sexual disorders, hormone-sensitive psychiatric and neurological diseases, and specific symptoms of diseases which present as a syndrome, such as McCune-Albright

IPR2023-00940 Page 00014

disease or polycystic ovarian syndrome. As with any diseases, the underlying causes of neuroendocrine dysregulation can be varied, and can be inherited (mono- or polygenic), acquired, associated with tumorigenesis and/or carcinogenesis, or otherwise associated with another medical condition. Diagnosis and treatment of diseases with neuroendocrine components must always take into account the multidimensional nature of the controlled neuroendocrine system. Diseases that cause dysregulation in the neuroendocrine homeostatic axes are considered *primary*, if they involve the major end-organ of the axis, or *secondary*, if defects occur at antecedent levels (pituitary or hypothalamus). Neuroendocrine diseases are usually diagnosed with the aid of X-rays, MRIs and other imaging diagnostic procedures, along with measurements of pituitary and end-organ hormones in serum. In some cases, provocative tests of a target-organ function are conducted with stimulatory hormone preparations.

THE STUDY OF NEUROENDOCRINOLOGY

The methods used to study neuroendocrine systems are as diverse as those employed by neuroscientists and endocrinologists across a full spectrum of investigative levels, from molecular biology to behavior. Classical *in vivo* and *in vitro* methods include surgical manipulations, hormone treatments, neuropharmacological approaches, immunohistochemical analysis of neuropeptide or hormone receptor expression, cell and tissue culture methods, radioligand receptor binding assays, *in situ* hybridization, quantitative real-time PCR, and immunoassays of hormones in body fluids and tissue extracts. Modern neuroendocrine research utilizes virtually any of the latest molecular, cellular, physiological, behavioral and genetic approaches — often in combination with the classical techniques — to further understanding of neurohormone synthesis, secretion, physiological roles and mechanisms of action, as well as the molecular and cellular actions of peripheral hormones on brain development and function.

There are inherent challenges in the study of neuroendocrine systems that derive from the heterogeneity of the cell groups in the hypothalamus, and the relative lack of stereotyped cell circuitries that are more easily identified and accessed in the hippocampus, cerebellum and other brain structures. Neuroendocrinologists have therefore often led the way in developing new methods to analyze the cellular and integrative properties of hypothalamic neurons, and their functional roles in physiological contexts. *Immortalized cell lines* producing the neurohormone GnRH were initially developed by Mellon *et al*.[32] and Radovick *et al*.[33] by targeted tumorigenesis, and these and subsequent cell lines have permitted sophisticated cell and molecular analyses of neuropeptide gene transcription, intra- and intercellular signaling mechanisms, and cellular substrates of neuronal migration. *Transgenic mouse lines* have been developed by neuroendocrinologists that have permitted targeted alteration or mapping of gene expression in specific subsets of hypothalamic cells. Transgenesis has been used to label cells of a certain phenotype, such that they may be visualized in the living state. For example, the promoter sequence for the GnRH gene was fused to a gene encoding the jellyfish green fluorescent protein (GFP) to create transgenic mice in which the transgene, and thus the fluorescent GFP molecule, is only expressed in GnRH neurons.[34,35] This animal model has been used to permit visualization of these few neurons in living brain tissues for electrophysiological experiments.

Gene knockout and *knockin* animals have provided a wealth of information about the physiological roles of hormones and their receptors in the central nervous system. The loss of function in gene-deletion mutant animals can confirm the essential roles of a protein in a specific neuroendocrine process, or it can even replicate a neuroendocrine disease state that is believed to arise from analogous gene mutations in humans. For example, the critical importance of the estrogen receptor α (ERα) in mediating estradiol feedback effects in the reproductive axis, sexual behavior and the neuroendocrine regulation of energy homeostasis has been firmly established using ERα null mutant mice.[36–38] Additional knowledge of the specific ERα signaling mechanisms mediating neural responses to estrogens has been provided by the study of ERα mutant gene knockin mice,[38,39] which can only exert effects through non-classical signaling mechanisms that do not utilize canonical geneotropic regulatory mechanisms. Similarly, an obligatory role of progesterone receptors in the stimulation of preovulatory gonadotropin surges was demonstrated through the study of progesterone receptor knockout (PRKO) mice.[40] Conditional gene targeting has likewise produced profound insights into the cellular and molecular physiology of neuroendocrine systems. In cell-specific gene targeting, the loxP—Cre recombination system is one such method involving the generation of mice bearing site-specific recombination sites, called loxP sites, in the intronic sequences that flank an essential exon of the target gene. A second line of mice is produced that harbor a transgene construct containing a promoter fused to the Cre recombinase gene; importantly, the promoter is known to be active only in the cells of interest. Mating of the two mouse lines results in the expression of Cre protein in the targeted cells, where it acts at the loxP sites to delete the exon of the target gene, thereby inactivating it. Thus, the specific gene

IPR2023-00940 Page 00015

deletion only occurs in the target cells. Many striking examples of the successful use of this system are found among studies that have produced cell-specific deletions of leptin-receptor or insulin-receptor genes in hypothalamic neurons, producing animals with altered energy homeostasis.[41,42] Targeted deletion of ERα in neurons that produce the reproductive neuropeptide, kisspeptin, was recently found to advance the onset and prevent the completion of puberty, unambiguously implicating these receptors in the neuroendocrine mechanisms that orchestrate reproductive maturation.[43]

SUMMARY

In this chapter, the science of neuroendocrinology has been introduced and general descriptions of neuroendocrine systems have been provided, with the intention of preparing the generalist for the detailed treatment of specific neuroendocrine topics in this book. The reader should now be acquainted with the functional anatomy and the basic operating principles of the major mammalian neuroendocrine systems. Intensive and up-to-date treatments of neuroendocrine signaling mechanisms, feedback mechanisms, neurohypophysial systems and hypothalamic–pituitary–end-organ axes, and the actions of hormones in the CNS, follow in subsequent chapters by renowned experts in the field.

References

1. Scharrer E, Scharrer B. Neurosecretion. *Physiol Rev.* 1945;25:171–181.
2. Schally AV. Aspects of hypothalamic regulation of the pituitary gland. *Science.* 1978;202(4363):18–28.
3. Guillemin R. Peptides in the brain: the new endocrinology of the neuron. *Science.* 1978;202(4366):390–402.
4. Houssay BA, Biasotti A, Sammartino R. Modifications functionelles de l'hypophyse après les lesions infundibulotuberiennes chez le crapaud. *C.R. Soc Biol Paris.* 1935;120:725–727.
5. Wislocki GB, King LS. The permeability of the hypophysis and the hypothalamus to vital dyes with a study of the hypophsyial vascular supply. *Am J Anat.* 1936;58:421–472.
6. Green JD, Harris GW. The neurovascular link between the neurohypophysis and adenohypophysis. *J Endocrinol.* 1947; 5:136–146.
7. Markee JE, Sawyer CH, Hollinshead WH. Activation of the anterior hypophysis by electrical stimulation in the rabbit. *Endocrinology.* 1946;38:345–357.
8. Harris GW. Electrical stimulation of the hypothalamus and the mechanism of neural control of the adenohypophysis. *J Physiol Lond.* 1948;107:418–429.
9. Vale W, Spiess J, Rivier C, Rivier J. Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotrophin and beta-endorphin. *Science.* 1981; 213(4514):1394–1397.
10. Guillemin R, Brazeau P, Bohlen P, Esch F, Ling N, Wehrenberg WB. Growth hormone releasing factor from a human pancreatic tumor that caused acromegaly. *Science.* 1982;218(4572):585–587.
11. Rivier J, Spiess J, Thorner M, Vale W. Characterization of a growth hormone-releasing factor from a human pancreatic islet tumour. *Nature.* 1982;300(5889):276–278.
12. Douglas WW, Poisner AM. Stimulus-secretion coupling in a neurosecretory organ: the role of calcium in the release of vasopressin from the neurohypophysis. *J Physiol.* 1964;172:1–18.
13. Lincoln DW, Wakerley JB. Electrophysiological evidence for the activation of supraoptic neurons during the release of oxytocin. *J Physiol.* 1974;242:533–554.
14. Yamashita H. Effect of baro- and chemoreceptor activation on supraoptic nuclei neurons in the hypothalamus. *Brain Res.* 1977;126:551–556.
15. Werner J. System properties, feedback control and effector coordination of human temperature regulation. *Eur J Appl Physiol.* 2010;109:13–25.
16. Levin BE. Developmental gene × environment interactions affecting systems regulating energy homeostasis and obesity. *Front Neuroendocrinol.* 2010;31(3):270–283.
17. Castaneda TR, Tong J, Datta R, Culler M, Tschop MH. Ghrelin in the regulation of body weight and metabolism. *Front Neuroendocrinol.* 31(1):44–60.
18. Sánchez-Lasheras C, Könner AC, Brüning JC. Integrative neurobiology of energy homeostasis-neurocircuits, signals, and mediators. *Front Neuroendocrinol.* 2010;31(1):4–15.
19. Niswender KD, Schwartz MW. Insulin and leptin revisited: adiposity signals with overlapping physiological and intracellular signaling capabilities. *Front Neuroendocrinol.* 24(1):1–10. Review.
20. Kaynard AH, Pau KY, Hess DL, Spies HG. Gonadotropin-releasing hormone and norepinephrine release from the rabbit mediobasal and anterior hypothalamus during the mating-induced luteinizing hormone surge. *Endocrinology* 1990; 27(3):1176–1185.
21. Arancibia S, Tapia-Arancibia L, Assenmacher I, Astier H. Direct evidence of short-term cold-induced TRH release in the median eminence of unanesthetized rats. *Neuroendocrinology.* 1983; 37(3):225–228.
22. Plotsky PM, Vale W. Hemorrhage-induced secretion of corticotropin-releasing factor-like immunoreactivity into the rat hypophysial portal circulation and its inhibition by glucocorticoids. *Endocrinology.* 114(1):164–169.
23. Rivier C, Rivier J, Mormede P, Vale W. Studies of the nature of the interaction between vasopressin and corticotrophin-releasing factor on adrenocorticotropin release in the rat. *Endocrinology.* 1984;115(3):882–886.
24. Schneider JE. Energy balance and reproduction. *Physiol Behav.* 2004;81(2):289–317.
25. Watanabe G, Terasawa E. *In vivo* release of luteinizing hormone releasing hormone increases with puberty in the female rhesus monkey. *Endocrinology.* 1989;125(1):92–99.
26. Harris GC, Levine JE. Pubertal acceleration of pulsatile gonadotropin releasing hormone release in male rats as revealed by microdialysis. *Endocrinology.* 2003;144(1):163–171.
27. McEwen BS, Wingfield JC. The concept of allostasis in biology and biomedicine. *Horm Behav.* 2003;43(1):2–15.
28. Levine JE, Pau KY, Ramirez VD, Jackson GL. Simultaneous measurement of luteinizing hormone-releasing hormone and luteinizing hormone release in unanesthetized, ovariectomized sheep. *Endocrinology.* 1982;111(5):1449–1455.
29. Belchetz PE, Plant TM, Nakai Y, Keogh EJ, Knobil E. Hypophysial responses to continuous and intermittent delivery of hypothalamic gonadotropin-releasing hormone. *Science.* 1978;202(4368):631–633.
30. Crowley Jr WF, Filicori M, Spratt DI, Santoro NF. The physiology of gonadotropin releasing hormone (GnRH) secretion in men and women. *Recent Prog Horm Res.* 1985;1985(41):473–531.

IPR2023-00940 Page 00016

31. Bouret SG, Draper SJ, Simerly RB. Trophic action of leptin on hypothalamic neurons that regulate feeding. *Science.* 2004; 304(5667):108–110.
32. Mellon PL, Windle JJ, Goldsmith PC, Padula CA, Roberts JL, Weiner RI. Immortalization of hypothalamic GnRH neurons by genetically targeted tumorigenesis. *Neuron.* 1990;5(1):1–10.
33. Radovick S, Wray S, Lee E, Nicols DK, Nakayama Y, Weintraub BD, Westphal H, Cutler GB, Wondisford FE. Migratory arrest of gonadotropin-releasing hormone neurons in transgenic mice. *Proc Natl Acad Sci USA.* 1991;88(8):3402–3406.
34. Spergel DJ, Krüth U, Hanley DF, Sprengel R, Seeburg PH. GABA- and glutamate-activated channels in green fluorescent protein-tagged gonadotropin-releasing hormone neurons in transgenic mice. *J Neurosci.* 1999;19(6):2037–2050.
35. Suter KJ, Song WJ, Sampson TL, Wuarin JP, Saunders JT, Dudek FE, Moenter SM. Genetic targeting of green fluorescent protein to gonadotropin-releasing hormone neurons: characterization of whole-cell electrophysiological properties and morphology. *Endocrinology.* 2000;141(1):412–419.
36. Ogawa S, Eng V, Taylor J, Lubahn DB, Korach KS, Pfaff DW. Roles of estrogen receptor-alpha gene expression in reproduction-related behaviors in female mice. *Endocrinology.* 1998;139(12):5070–5081.
37. Park CJ, Zhao Z, Glidewell-Kenney C, Lazic M, Chambon P, Krust A, Weiss J, Clegg DJ, Dunaif A, Jameson JL, Levine JE. Genetic rescue of nonclassical ERα signaling normalizes energy balance in obese Erα-null mutant mice. *J Clin Invest.* 2011;121(2):604–612.
38. Glidewell-Kenney C, Hurley LA, Pfaff L, Weiss J, Levine JE, Jameson JL. Nonclassical estrogen receptor alpha signaling mediates negative feedback in the female mouse reproductive axis. *Proc Natl Acad Sci USA.* 2007;104(19):8173–8177.
39. McDevitt MA, Glidewell-Kenney C, Jimenez MA, Ahearn PC, Weiss J, Jameson JL, Levine JE. New insights into the classical and non-classical actions of estrogen:evidence from estrogen receptor knock-out and knock-in mice. *Mol Cell Endocrinol.* 2008;290(1-2):24–30.
40. Chappell PE, Schneider JS, Kim P, Xu M, Lydon JP, O'Malley BW, Levine JE. Absence of gonadotropin surges and gonadotropin-releasing hormone self-priming in ovariectomized (OVX), estrogen (E2)-treated, progesterone receptor knockout(PRKO) mice. *Endocrinology.* 1999;140(8):3653–3658.
41. Brüning JC, Gautam D, Burks DJ, Gillette J, Schubert M, Orban PC, Klein R, Krone W, Müller-Wieland D, Kahn CR. Role of brain insulin receptor in control of body weight and reproduction. *Science.* 2000;289(5487):2122–2125.
42. Ring LE, Zeltser LM. Disruption of hypothalamic leptin signaling in mice leads to early-onset obesity, but physiological adaptations in mature animals stabilize adiposity levels. *J Clin Invest.* 2010;120(8):2931–2941.
43. Mayer C, Acosta-Martinez M, Dubois SL, Wolfe A, Radovick S, Boehm U, Levine JE. Timing and completion of puberty in female mice depend on estrogen receptor alpha-signaling in kisspeptin neurons. *Proc Natl Acad Sci USA.* 2010;107(52):22693–22698.

IPR2023-00940 Page 00017