# Neuroendocrine Integration of Body Weight Regulation

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## Introduction

The brain plays a critical role in the regulation of physiological processes including energy homeostasis. Central nervous circuits instantly assess and integrate peripheral metabolic, endocrine and neuronal signals, and coordinate a response that modulates both behavioral patterns and peripheral metabolism according to acute and chronic requirements ( 1 ). The brain directs, coordinates and integrates circulating hormones and metabolites that signal energy availability. As a consequence it modifies energy intake and expenditure to match energy demands on an ongoing homeostatic basis, establishing a metabolic “set-point”. This point is also strongly influenced by central neural circuits that regulate motivation, hedonism, reward and liking of food. The impact of the non-homeostatic “hedonistic” aspects of food intake is now increasingly recognized, highlighted by the fact that animals and most humans in western societies exposed to a highly palatable diet overeat because they “like” to eat (hedonism), rather than “need” to eat (homeostasis).

Changes in body weight reflect respective changes in energy balance resulting from an imbalance between energy taken up (food intake) and energy expended (for locomotor activity, basal metabolism, and thermogenesis). Therefore, any alterations in food intake or energy expenditure precede measurable alteration in body weight. This chapter describes the different components of the central neural control of body weight, including homeostatic regulation and “higher” regulatory systems, i.e. non-homeostatic pathways. In addition, the neuroendocrine regulation of food intake is described considering that the net effect of respective changes in food intake and energy expenditure alters body weight. Further details and components considering the regulation of energy intake and expenditure as well as food intake are discussed in chapters 3, 4 and 7.

## How did it all start – a short historical excursion

The development of hunger as a physiological corollary in response to extended periods of food deprivation is a primal motivated behavior and comprises one of the most fundamental responses in all animals. The counterpart of this is satiation, which signals the termination of an ingestive behavior (food or water intake), that is equally important to the survival of animals. Historically, the biological basis of hunger and satiety has received considerable experimental attention, with the role of the brain as the main regulator in the control of food intake maintaining a central focus.

In 1954 Eliot Stellar suggested the concept of a “satiety center” situated in the ventral medial hypothalamus and a “hunger center” centered in the lateral hypothalamus ( 2 ). Stellar based these hypotheses on lesion studies. Rats with lesions of the “satiety center”, in the ventral medial hypothalamus, showed a marked increase in food intake whereas electrical stimulation of this area decreased food intake. Lesions of the opposing “hunger center” in the lateral hypothalamus showed decreased food intake whereas stimulation of this area increased food intake.

In the 1950s, two hypotheses for the generation of hunger dominated research into underlying brain mechanisms. The first, put forth by Mayer, stated that decreases in glucose utilization stimulated eating and increases in glucose utilization halted eating, with the combined effect being referred to as the “glucostatic hypothesis” of hunger ( 3 , 4 ). Supporting this hypothesis, two types of glucose sensitive neurons were identified changing their action potential in dependence from surrounding glucose levels. Glucose excited (GE) neurons increase activity and glucose inhibited (GI) neurons decrease activity with rising glucose levels. Intrahypothalamic glucose administration decreases food intake and inhibits hepatic glucose production, suggesting that glucose sensing neurons indeed may regulate appetite and blood glucose concentrations ( 5 ).The second hypothesis, promulgated by Kennedy, proposed that fat mass produced an inhibitory control of eating and body weight gain, with the inhibitory control mediated by an unidentified humoral signal from white adipose tissue acting on the ventral medial hypothalamus. Thus, food intake increased after lesions of the ventral medial hypothalamus due to the removal of the site of action of the inhibitory signal from the fat. This formed the basis of the “lipostatic hypothesis” of hunger . The inhibitory agent central to the lipostatic hypothesis was later identified as leptin ( 6 ). The discovery of the so called *ob* gene that carries the genetic information for the adipose tissue-derived factor leptin represents the most influential milestone in the field of obesity research to date and forms a backdrop to the study of homeostatic mechanisms and modulation of food intake that has dominated the field for the last two decades.

# How does the brain work? – A brief anatomical excursion

The main information processing units of the brain are neurons. Neurons are cells of ectodermal origin that have subcellular compartments specialized in receiving (dendrites and perikaryon) and forwarding (axons) information. Neurons interact with each other by synapses that are established between axon terminals and dendritic or perikaryal membranes. The information travels from the axon terminals to the dendritic or perikaryal membranes. Synaptic transmission occurs either electrically, chemically via release of neurotransmitters or (neuro-) modulators

from synaptic vesicles of the axon terminal or by release of gaseous substances such as nitric oxide or carbon monoxide. In most cases, synaptic transmission can occur by all three means, although one may predominate depending on the action potential as well as on substances that may directly signal to the axon terminal from the extracellular space (such as other neuromodulators released by nearby). The specificity of signal transduction is ensured by the appropriate connectivity within a given network and by the availability of receptors at specific sites for released neurotransmitters/modulators or peripheral metabolic hormones. It is rather likely that interaction between neurons holds the key to understanding metabolism regulation. However, emerging data clearly suggests that glial cells also play a pivotal role in allowing normal neuronal activity by providing enzymatic activity or substrates leading to changes in activity of adjacent neurons ( 7-9 ) To this end, it is critical that the principles of neuronal physiology are mastered (beyond the short references provided here) to the deepest possible extent. Otherwise, attempts to decipher the role of the brain in obesity research will remain elusive.

# Communication via neurotransmitters

Neurotransmitters belong to a heterogeneous class of signal molecules that transmit the signal of impaired neuronal activation from one neuron to the other. This transmission is thought to happen mainly at synaptic endings of neurons. Impaired activation potential of a neuron leads to secretion of neurotransmitters into the synaptic cleft and binding to corresponding receptors that sit on the opposite, postsynaptic membrane of the adjacent neuron. This process changes the action potential of this neuron and so forth. Among neurotransmitters are several neuropeptides (e.g. α-melanocyte stimulating hormone (α-MSH), neuropeptide Y (NPY), orexins), biogenic amines (e.g. acetylcholine, serotonin, dopamin, histamine, epinephrine, norepinephrine), amino acids (e.g. glutamate, γ aminobutyric acid (GABA), aspartatic acid, glycine) and gases (e.g. nitric oxide (NO), carbon monoxide (CO)). The most important neurotransmitters in the brain are glutamate and GABA. They play important roles in regulating neuronal activity in the hypothalamus and therefore contribute significantly to the central regulation of energy balance.

*GABA* represents the main inhibitory neurotransmitter in the central nervous system (CNS) ( 10 ). Intracerebroventricular injections of GABA induce feeding and positive energy balance. Intriguingly, immunoreactivity of glutamic acid decarboxylase (GAD), the enzyme that catalyzes the final step of GABA synthesis (used as marker for GABAergic neurons), co-localizes to a high percentage with orexigenic neurons (NPY/agouti-related protein (AgRP) expressing) in the ventromedial part of the arcuate nucleus (ARC) ( 10 ). But also one third of anorexigenic neurons (proopiomelanocortin (POMC) expressing) were shown to contain GAD mRNA and those neurons are able to release GABA. GABA receptors were detected in hypothalamic regions that are important for the regulation of feeding.

*Glutamate* represents the main excitory neurotransmitter in the hypothalamic neuroendocrine regulation ( 10 ). Released glutamate is transported into adjacent neurons or glia cells by cell membranes located high-affinity excitatory amino acid transporters (EAAT). Inside the neuron, glutamate is transported into synaptic vesicles by vesicular glutamate transport proteins (VGLUT) that serve as markers for glutamergic neurons. Among the three known VGLUTs,

VGLUT2 represents the dominant hypothalamic glutamate transporter ( 10 , 11 ). Its co-localization with POMC/cocain-and amphetamine-related transcript (CART) neurons in the arcuate nucleus suggests that some POMC/CART neurons are glutamergic. In addition, glutamate may significantly contribute to the regulation of hypothalamic-pituitary-adrenal/thyroid axis, because 1) glutamatergic neurons (possibly of the POMC/CART type) were suggested to heavily innervate hypophysiotrophic corticotropin-releasing hormone (CRH) and thyrotropin-releasing hormone (TRH) neurons in the paraventricular nucleus PVN ( 12 ) and 2) hypophysiotropic CRH and TRH neurons themselves were reported to be glutamatergic ( 13 ). Also hypothalamic somatostatin, vasopressin and oxytocin neurons have been reported to be of glutamatergic phenotype ( 14 , 15 ).

## Homeostatic regulation of energy balance: connecting the hypothalamus, hindbrain and the gut.

Homeostatic regulation of energy balance requires the brain to maintain the appropriate energy levels by instantly modulating metabolites, fuel stores or hormone secretion. This demands first the ability of sensing metabolic and hormonal changes in the periphery by integrating the information from afferent signals projecting to the brain. Those signals include tissue-specific signaling molecules (e.g. leptin, ghrelin, cholecystokinin), metabolites (e.g. glucose, fatty acids), hormones (e.g. insulin, glucocorticoids, adrenaline, noradrenaline) or vegetative nerve terminals (e.g. vagal afferents). Peptides secreted by the gastrointestinal tract during eating or digestion mainly act as satiety signals, conveying information on short-term changes in nutrient and energy supply, whereas factors secreted by other peripheral organs and tissues (e.g. adipose tissue, pancreas) rather serve to signal long-term changes in metabolic state or energy stores. In addition, hormones reflecting caloric intake and acute nutritional requirements complete the information flow to the brain (see detailed description of afferent signals below).

Inside the brain, central nervous circuits coordinate a respective efferent response that modulates both behavioral patterns and peripheral metabolism according to acute and chronic requirements ( 1 ).

The classical endocrine axes, consisting of hypothalamic releasing hormones, pituitary hormones and peripheral endocrine signals are, without exception, involved in mediating efferent information and maintaining the balance of metabolism and energy homeostasis.

The hypothalamo-pituitary-adrenal (HPA) axis with corticotropine releasing hormone (CRH), adrenocorticotropic hormone (ACTH) and **corticosteroids** is mediating stress responses, contributes to glucose mobilization and induces a positive energy balance. The hypothalamo-pituitary-thyroid (HPT) axis with thyreotropin releasing hormone (TRH), throidea stimulating hormone (TSH) and **thyroid hormones** (triiodothyronine (T3) and thyroxine (T4)) is an important stimulator of development and metabolism. In case of demand, T4 is tissue-specifically converted into the active thyroid hormone T3 mainly by type 1 and 2 deiodinase whose expression pattern and activity play an additional role in mediating thyroid hormone effects ( 16 ). Thyroid hormones increase metabolic rate and produce energy deficits. Their circulating level correlates directly with basal metabolic rate. The **growth hormone (GH)-**

**insulin-like growth factor 1 (IGF-1) axis** (somatotropic axis) regulates growth and metabolism. GH secretion from the pituitary is induced by hypothalamus derived growth hormone releasing hormone (GHRH). GH directly promotes lipolysis and glycogenolysis, while IGF-1 stimulates protein synthesis (muscle growth), cell division and bone growth. The hypothalamo-pituitary-gonadal axis with hypothalamus-derived gonadotropin releasing hormone (GnRH), the pituitary-derived hormones luteinizing hormone (LH) and follicle stimulating hormone (FSH) and the **gonadal hormones** testosterone (secreted from testis) and estrogens and gestagens (e.g. estradiol and progesterone secreted from ovaries) regulates functions necessary for reproduction. Since reproduction is dependent on sufficient energy stores gonadal hormones also regulate energy balance. Testosterone mainly acts in an anabolic pattern, stimulating protein synthesis and increasing the ratio of muscle to fat mass in men. Progesterone has mainly orexigenic functions, and estradiol is anorexigenic causing a negative energy balance in women. Although classical hormones secreted by the endocrine glands are largely neglected as afferent signals that convey peripheral metabolic states, energy stores or nutritional requirements to the brain, they play an important role in the complex networks governing appetite and body weight ( 17 ). Central administration of **corticosteroids** , for example, induces appetite and increases fat mass ( 18 ). While the negative energy balance induced by **thyroid hormones** is mainly attributed to their multiple peripheral effects, T4 as well as T3 receptors are also localized in the brain, where they serve as important feedback targets ( 19 ). The same feedback principle exists for hormones of the somatotropic axis such as growth hormone (GH) and insulin-like growth factor I (IGF-I) ( 17 ).

The other effector system is the autonomous nervous system (the parasympathetic and the sympathetic nervous system) that is believed to represent the predominant efferent pathways from the brain to the periphery in the adjustment of energy balance ( 20 , 21 ) See also http://www.endotext.org/obesity/obesity4/obesityframe4.htm for more detailed information.

There has always been a trend to simplify the regional contribution of brain areas to homeostatic regulation of energy balance: It allows a relatively simple conceptualization of the involvement of different brain mechanisms. It also contributes to a rapid advancement in knowledge of the involvement of particular neuropeptides and their receptors in these processes. It is important to remember that despite ever increasing data gathered in the last century, including the discovery of leptin and the repeated revelations of “the most important” hormone or neuropeptide, an effective pharmacotherapy for obesity is still lacking. Thus, when considering the brain structures discussed below and their putative function, the reader is advised to keep in mind that this is a reflection of our current understanding and not necessarily a rigid blueprint of metabolic regulation. Our present knowledge of these circuits continues to improve, but is far less clear than the pathways for vision, hearing or basic motor functions that, for example, involve and require interaction between the cortex, basal ganglia, striatum and cerebellum.

## Where is it happening? The main brain areas of homeostatic regulation

Two of the major brain areas that play a key role in the homeostatic regulation of energy balance are the hypothalamus (including the arcuate nucleus, ARC) and the brainstem

(including the nucleus tractus solitarus, NTS). Both of these have strong connections to circumventricular organs (e.g. median eminence, area postrema) that lack a normal blood brain barrier or contain specialized transport systems to allow the direct access of peripheral signaling molecules, nutrients, metabolites and hormones ( 22 ) that therefore cannot access other brain areas. The hypothalamus is considered the main integrator and processor of peripheral metabolic information especially for factors signaling medium-term to long-term changes in energy balance like leptin, insulin and ghrelin ( 23-33 ). This basal diencephalic area of the brain contains several nuclei (i.e. collection of neuronal cells) that have been shown in the past century to be key brain regions in the regulation of homeostasis. Depending on the metabolic status signal integration in hypothalamic nuclei mainly contributes to meal initiation ( 34 ). The brainstem on the other hand, integrates short-term signals, mainly originating from the gastrointestinal tract contributing to meal termination (e.g. cholecystokinine and neuronal input from vagal afferents) ( 34-36 ).

## Who tells the brain what‘s going on in the body? (afferent signals)

Since the discovery of leptin much attention has been focused on the arcuate nucleus in the mediobasal hypothalamus, the central “relay hub” which integrates peripheral metabolic signals. A current classification of signals involved in the regulation of energy balance differentiates between 1) adiposity signals (i.e. leptin and insulin) that are secreted in proportion to body fat stores, 2) satiety signals (i.e. cholecystokinin (CCK); glucagon-like peptide 1 (GLP-1)) which are gastrointestinal peptides, that are secreted in association with meals indicating caloric quantity and quality to the brain and 3) nutrient-related signals (i.e. glucose, free fatty acids, amino acids) generated through the ingestion of a meal. Nutrient-related signals directly inform centrally located sensors about the current state of carbohydrate and lipid metabolism ( 37 ).

### *Adiposity signals*

*Leptin* is the most prominent adipocyte hormone; its discovery in 1994 changed the by then existing concepts of energy balance regulation dramatically. The *ob/ob* mouse phenotype results from a spontaneous mutation in the leptin gene, that long before its identification, was already speculated to be caused by the lack of a peripheral signal that informs the brain about existing energy stores ( 38 ). Leptin is predominantly produced by fat cells and is expressed according to the size of fat stores ( 39 ). Its administration induces a negative energy balance mediated by neuronal structures in the hypothalamus and the brainstem (s. also following paragraphs).

The role of leptin in signaling the brain about chronic changes in energy status is completed by the pancreas derived hormone *insulin* , which conveys additional information about long-term changes of peripheral metabolism to the brain. Centrally administered insulin triggers a negative energy balance, while neuron-specific deletion of its receptor causes obesity ( 40 ). Central actions of insulin on energy balance are tightly linked to regulation of glucose homeostasis, the classical systemic function of insulin. By decreasing food intake insulin contributes to decrease blood glucose concentrations. In addition, insulin acts centrally to decrease hepatic glucose output, a mechanism, which is mainly mediated by AgRP neurons and vagal output to the liver (

41 , 42 ).

*Adiponectin* is almost exclusively expressed in adipose tissue. The protein consists of differently sized complexes ranging from homotrimers, constituting the minimal unit, to hexamers (low-molecular weight complex, LMW) and 16-18mers (high-molecular weight complex, HMW) ( 43 ). Unlike most other adipokines, adiponectin plasma levels and mRNA expression are decreased in obesity ( 43 ). Therefore, adiponectin is inversely correlated with BMI and with fat mass. Adiponectin levels rise during fasting and decrease during refeeding. In addition, adiponectin deficiency is associated with insulin resistance, glucose intolerance and hyperlipidemia. Adiponectin receptors have been identified in peripheral tissues (mainly skeletal muscle and liver), but were also detected in several areas of the brain, e.g. in the nucleus arcuatus and in the paraventricular hypothalamus ( 44 ). However, a possible central adiponectin action was questioned, because HMW adiponectin was not able to cross the blood brain barrier, while trimers and LMW adiponectin could be detected in cerebrospinal fluid following intravenous adiponectin injection ( 45 ). It was suggested that fasting-induced increased adiponectin levels activate hypothalamic AMP-activated protein kinase (AMPK) leading to positive energy balance by increasing food intake and decreasing energy expenditure. Therefore, during refeeding, decreasing adiponectin levels reverse this metabolic state towards decreased food intake and increased energy expenditure ( 44 ). This way, plasma adiponectin may be inversely regulated in relation to plasma leptin, and adiponectin was proposed to act as starvation signal, while leptin is regarded as satiety signal ( 44 , 46 ) emerging from the same tissue. However, others reported intracerebroventricular adiponectin administration to decrease body weight mainly by increasing energy expenditure ( 47 ). Thus, the impact of centrally acting adiponectin still needs to be elucidated.

*Resistin* was named for the ability to induce insulin resistance. In rodents, resistin levels are regulated similar to leptin levels: they increase during obesity, they decrease during fasting and rise again during refeeding ( 45 ). Resistin levels are regulated by insulin and glucose, a process which is obviously mediated by glucose-dependent insulinotropic polypeptide (GIP, 45 ). Resistin inhibits adipogenesis but leads to insulin resistance mainly by blunted insulin-induced suppression of hepatic glucose production. While the receptor for resistin is not known so far, resistin administered into the lateral ventricle acutely decreased food intake in rats ( 48 ) and induced hepatic insulin resistance in mice via increased expression of NPY ( 49 ). While expression of resistin mRNA (in ARC, ventromedial hypothalamus (VMH) and hippocampus, 48 ) and protein ( 50 ) have been reported in the brain, the significance of centrally acting resistin, either produced in the brain or entering from the periphery, is still unknown.

*Interplay of adiposity signals* . Considerable progress has been made in understanding the interaction and signaling pathways of neurons in the ARC since the identification of leptin and its receptor ( 51 ). Leptin signaling in the ARC is mediated via binding of leptin to the “long” (signaling) form of the leptin receptor (Ob-Rb) and stimulation of the tyrosine kinase JAK2 to phosphorylate STAT3 (signal transducer and activator of transcription 3) ( 52-55 ). Similarly, insulin receptors are expressed in the ARC. The insulin receptor recruits insulin receptor substrates (IRS) which are phosphorylated by the activated insulin receptor. In some cells leptin can also activate the insulin receptor substrate pathway, demonstrating one point of convergence and synergism between intracellular signaling pathways used by insulin and leptin

( 56 ). It was reported that intracellular signaling of leptin and insulin converge at the stage of phosphoinositide 3-kinase (PI3K) activation. However, it was suggested that the presence of insulin is necessary for leptin induced PI3K activation constituting a mechanism of an insulin-dependent sensitization of leptin action ( 57 ). Another receptor located in the ARC is the ghrelin receptor (growth hormone secretagogue receptor) ( 25 , 58 ). These and other receptors and/or their peptide ligands that are involved in homeostatic control of feeding are also located in the brainstem including melanocortin-4 receptors and leptin receptors ( 59-61 ).

## The arcuate nucleus – relay-hub and point of first contact

The ARC contains the so called “first order neurons” that mediate and integrate the first contact of peripheral signals with the CNS. There are two distinct sets of hypothalamic neurons situated in the arcuate nucleus having opposing effects on food intake ( 24 , 62 , 63 ). One population of neurons in the ARC co-expresses the anorexigenic precursor peptide proopiomelanocortin (POMC) as well as cocaine-and amphetamine-related transcript (CART); the other co-expresses the orexigenic peptides neuropeptide Y (NPY) and agouti-related protein (AgRP). A majority of both POMC/CART and NPY/AgRP neurons have been found to co-express leptin receptors (Ob-Rb mRNA) ( 24 , 63 ) and both types of neurons are regulated by leptin in an opposing manner ( 64 , 65 ). As such, administration of exogenous leptin activates POMC/CART neurons, as evidenced by increased POMC expression, increased signal transducer and activator of transcription 3 (STAT3) translocation, induced expression of Fos (marker of neuronal activity) and increased expression of suppressor of cytokine signaling-3 (SOCS-3) mRNA (cellular marker of direct leptin action) ( 62 , 66-68 ). Conversely, NPY/AgRP neurons are inhibited by leptin, an action that can be demonstrated by expression of SOCS-3. The same inhibitory effect on NPY/AgRP can be seen under insulin surge that also activates these neurons, since the ARC exhibits a high expression of insulin receptors ( 69 , 70 ). Reduced leptin and insulin levels on the other hand inhibit POMC and CART expression in the ARC ( 71 , 72 ). The net result is that conditions of starvation being commensurate with low levels of leptin (and insulin) result in promotion of the activity of NPY/AgRP neurons and the inhibition of POMC/CART neurons( 62 , 68 , 73-76 ).

## The CNS melanocortin system – the main essential system in homeostatic regulation

As noted above, POMC is a polypeptide precursor that is posttranslationally and site-specifically cleaved into several products, called melanocortins, including adrenocorticotrophic hormone (ACTH), α-melanocyte-stimulating hormone (α-MSH, more prominent in rodents), ß-MSH (more prominent in humans) and γ-MSH, and into ß-endorphin. ( 77 ). The central melanocortin system is a collection of CNS circuits that include 1) hypothalamic neurons originating in the ARC expressing NPY and AgRP or POMC, 2) brainstem POMC neurons originating in the nucleus tractus solitarius (NTS) and 3) downstream targets of these AgRP and POMC neurons that express the melanocortin-3 (MC3R) and melanocortin-4 receptor (MC4R) ( 77 ). While melanocortins function as endogenous agonists at the MC3/4R, AgRP works as highly efficient antagonist (inverse agonist) at these receptors.

MC3/4Rs are G-protein coupled receptors that are present throughout the brain, and are abundant particularly in the paraventricular nucleus (PVN), lateral hypothalamic area (LHA), dorsomedial hypothalamus (DMH) and ARC (only MC3R expressed at this location) ( 60 ). Stimulation of MC3/4 receptors induces a negative energy balance by decreasing food intake and increasing energy expenditure in animals and humans ( 78-80 ). These effects are presumably accomplished by separate □-MSH and AgRP neurons projecting to different MC4R expressing neurons. MC4R expressed in the PVN and/or in the amygdala regulate melanocortin-mediated food intake and body length. Melanocortin-mediated regulation of energy expenditure instead is obviously achieved by other MC4R-containing areas ( 81 ).

AgRP by acting as inverse agonist at MC3/4R decreases receptor activity and induces positive energy balance by increasing food intake and decreasing energy expenditure. The NPY/AgRP system not only antagonizes anorexigenic melanocortin cells at their target sites via MC4 receptors, but it also provides an anatomical unidirectional projection to POMC/CART neurons, that inhibits POMC cells robustly and directly via synaptic release of the inhibitory transmitter γ–aminobutyric acid (GABA) ( 68 ). This interaction provides a chronic inhibition of the POMC expressing neurons whenever NPY/AgRP neurons are active. The melanocortin system constitutes the core of homeostatic energy regulation and its activity is modified by both peripheral and neuronal signals. Its importance for the regulation of energy balance is underlined by the fact that estimated 2% of the obese population in Europe carries pathogenic mutations in the MC4 receptor ( 82 ).

## The lateral hypothalamic/perifornical system – the basis for Eliot Stellar's hunger center ; beyond the arcuate hub

Numerous observations support the previously described model of metabolic signals (i.e. leptin, insulin, gut hormones) acting on the two subsets of neurons with opposing effects on feeding in the ARC (first order neurons). These either stimulated or inhibited neurons in turn activate either orexigenic or anorexigenic peptide-expressing neurons in other nuclei of the hypothalamus (second order neurons) that will exert opposing effects on food intake and energy balance. One of these important regions is the lateral hypothalamic area (LHA)

Neurons in the LHA, secrete the orexigenic peptides melanin-concentrating hormone (MCH) ( 83 ) and orexins (also called hypocretins) ( 84 ) which are involved in feeding and arousal. MCH- and orexin-expressing neurons modulate a wide array of functions including behavioral responses to memory, learning, emotion and motivation, and motor responses in association with changes in energy state ( 85-88 ). Several metabolic signals including leptin and the gut hormone ghrelin activate both sets of LHA neurons ( 89 , 90 ).

Several studies have shown morphological connections between orexin fibers and NPY neurons in the ARC with orexin terminals making direct synaptic contact with neurons that express both, NPY and leptin receptors ( 91 ). Orexin stimulates food intake when injected centrally – upon fasting its precursor mRNA is upregulated ( 84 ) and orexin neurons are rapidly activated. Elevated levels of orexins not only elevate food intake, but also induce arousal and adiposity. In contrast, MCH shows little or no interaction with NPY neurons in the ARC, but shows

characteristic orexigenic properties. Similarly, administration of MCH causes an increase in food intake and body weight gain and decrease in energy expenditure at the same time ( 83 ). Two current genetic mouse models, the MCH knock out mouse, as well as the MCH neuron ablated model, show reduced food intake and increased energy expenditure ( 92 , 93 ). MCH mRNA is over-expressed in the obese *ob/ob* mouse model, and fasting further elevatesMCH levels in food-deprived normal and obese mice ( 83 ). The expression of the orexigenic neuropeptides MCH and orexin display the base of Stellars hunger center underlining their importance for energy balance regulation.

As will be outlined below, the LHA has and additional important function in mediating and integrating the interaction between homeostatic and non-homeostatic regulatory systems inside the CNS.

## Other hypothalamic centers involved in the homeostatic regulation of energy balance

Apart from the LHA, the PVN, the ventromedial hypothalamic nucleus (VMH) and DMH also play a critical role in modulating energy balance. Neurons in the PVN have a contrasting profile (anorexigenic) to those in the LHA and express hormones including corticotropin releasing hormone (CRH), thyrotropin releasing hormone (TRH) and oxytocin with anorexigenic properties as well as both MC3/4R and various NPY receptors (Y receptors) ( 94-97 ).

Stimulation of the PVN inhibits food intake, and bilateral lesions cause a hyperphagic obesity syndrome. Administration of CRH, TRH and oxytocin result in a reduction in food intake, while TRH in addition stimulates the thyroid axis and oxytocin regulates uterine function ( 94-96 , 98 , 99 ). The VMH has anorexigenic properties and increases energy expenditure as demonstrated by chemical lesion studies ( 100 , 101 ). It represents the basis for Stellars satiety center( 2 ). The VMH is also a region with high expression of the long form of the leptin receptor. However, to date little is known regarding the cellular mechanisms by which neurons in the VMH control energy balance. The DMH has long been implicated in energy balance regulation, apart from being involved in modulation of, for example, body temperature, arousal and circadian rhythms of locomotor activity ( 102 ). This nucleus expresses leptin receptors and receives direct input from the ARC ( 103 , 104 ). A recent study demonstrated its critical role in the entrainment of circadian rhythms to feeding schedules ( 105 ). However, this function is still under debate ( 106 ). The nature of cells within this nucleus remains topical but poorly defined.

In summary, neurons in the arcuate nucleus and lateral hypothalamus act as integrative metabolic sensors, generating output signals that drive the endocrine, autonomic and behavioral systems, and controlling energy intake and expenditure in a coordinated fashion to determine body weight.

## More about satiety signals: What makes us stop eating? How do we know when enough is enough? The importance of gut-brain interactions

How is energy homeostasis regulated on a meal to meal basis? Obviously both the amount of food consumed during each individual meal and the frequency of meals must be kept in balance. The onset of satiety seems to be the major determinant of meal size. What signals transport and translate information about satiety to the brain and which pathways are involved in response to these so-called satiety signals? As discussed above the hypothalamus, in particular the mediobasal portion, is the main mediator of the adiposity signals leptin and insulin; however, satiety information generated during the course of a meal seems to be largely communicated to the hindbrain via afferent fibers of the vagus nerve originating in the gastrointestinal tract. Satiety signals (i.e. CCK, GLP-1, amylin) have a major effect in decreasing meal size. Satiety-inducing signals can be activated and secreted in response to either mechanical or chemical stimulation of the stomach or small intestine during ingestion of food. Many of the signals provide information to the brain that influence energy homeostasis either directly by penetrating the blood-brain-barrier and interacting with specific neuronal receptors, or indirectly by stimulating neurons that in turn forward signals to the brain.

Mechanoreceptors lining the gastrointestinal tract are activated during ingestion and digestion of food involved in the “short-term” regulation of feeding, thought to play a major role in satiation. The gastric distension-sensitive vagal afferents are mechanosensors that increase activity in relation to increasing gastric volume but do not seem to provide much information about the chemical composition or nutrient content. In contrast, vagal afferents innervating the duodenum respond to the mechanical properties as well as the chemical characteristics of duodenal nutrients. Potential tension sensors (stretch receptors) are distributed throughout the gastrointestinal tract including the colon. In addition, vagal sensory terminals penetrate the mucosa of the entire gastrointestinal tract, where they are likely to detect chemical and mechanical stimuli ( 107 , 108 ). Vagal afferent neurons express receptors for several gastrointestinal hormones including CCK, ghrelin and GLP-1 ( 109 , 110 ). Mechanisms involving mechanoreceptors as well as gastrointestinal signals are also discussed in chapter 3.

Signals derived from the gastrointestinal tract are transported via vagal afferent fibers that synapse in the nucleus NTS in the hindbrain, which participates in gustatory, satiety and visceral sensation ( 111-113 ). All but two of these signals cause meals to terminate. The only peripheral signal to potentially stimulate food intake besides adiponectin, is the stomach hormone ghrelin that is normally secreted in anticipation of a meal and can therefore contribute to meal initiation.

*Cholecystokinin* is the most well established gut derived satiety signal. It is an acutely acting signal with a very short half-life which is secreted from neuroendocrine secretory cells lining the intestinal lumen in response to nutrients in chime ( 114 ). Early studies showed that exogenous CCK administered just prior to a meal reduced food intake in rats. In the last thirty years these results have been repeated and extended in numerous labs, which have demonstrated that the anorectic effects of CCK can be translated to virtually all species, including humans ( 115-117 ). CCK-1 receptors are expressed on sensory fibers of the vagus nerves innervating the tissue around the pyloric sphincter and the proximal duodenum ( 118 , 119 ). CCK can either act directly on the sensory fibers of the vagus nerve or via other branches of the nerve at the stomach wall that are activated via gastric distension through the meal bolus. Its signal is then transported via the hindbrain where it can initiate local reflexes or relayed to the forebrain (

120-123 ).

*Glucagon-like peptide 1 (GLP-1)* is synthesized in L-cells of the distal small intestine as well as in the brainstem in response to the appearance of nutrients in the small intestine (esp. of glucose or free fatty acids) ( 124 ). It increases insulin secretion during meals (incretin function) and has an anorexigenic effect similar to CCK. Intriguingly, appropriate intestinal GLP-1 secretion has been reported in dependence of the functional sweet taste receptor □-gastducin ( 125 ). The receptors that trigger the satiety action of centrally injected GLP-1 are located in the hypothalamus and the brainstem ( 126 ), whereas GLP-1 receptors that regulate glucose tolerance are located in the nodose ganglia and nerve terminals innervating the portal vein ( 127 ). Both, central and systematic administration of GLP-1 reduces food intake in rats as well as humans and causes hypophagia and weight loss ( 128-130 ). Caudal brainstem processing is obviously sufficient for mediating the suppression of intake, core temperature, and gastric emptying rates as well as tachycardia triggered by peripheral GLP-1R activation and also hindbrain-delivered ligand. It was suggested that hypothalamic/forebrain processing and forebrain-caudal brainstem communication is not required for the observed responses ( 131 ). Centrally acting GLP-1 was reported to decrease lipid accumulation in white adipose tissue, an effect that was independent from changes in food intake and partially mediated by the sympathetic nervous system ( 132 ). However, incretin function was suggested to be mainly mediated by binding and activating sensory afferent neurons originating in the nodose ganglion, which may in turn activate neurons of the NTS ( 124 ). Ascending fibers from NTS neurons may induce reflexes in the hypothalamus and descending impulses in turn may activate vagal motor neurons that send stimulatory or inhibitory impulses to the pancreas and the gastrointestinal tract ( 124 ). Since half-life of GLP-1 is extremely low due to high dipetidylpeptidas IV activity, this activation of sensory neurons seems to be more important under physiological conditions than stimulation of insulin secretion induced by direct GLP-1 receptor binding to the □-cell ( 124 ).

*Intestinal glucose-dependent insulinotropic polypeptide* (GIP, formerly gastric inhibitory polypeptide) is secreted from K-cells in the duodenum and proximal jejunum as well as in the hippocampus in response to food intake ( 133 , 134 ). Similarly to GLP-1, intestinal GIP acts as an incretin by increasing glucose-dependent insulin release from pancreatic β-cells and therefore, it contributes to postprandial plasma glucose normalization. It is suggested that the incretin function of GIP is mediated either directly via pancreatic receptor activation ( 135 ) or via activation of non-ganglionic cholinergic neurons that innervate the islets, presumably as part of an enteric-neuronal-pancreatic pathway ( 136 ). However, since circulating GIP is similarly to GLP-1 exposed to fast and efficient inactivation by dipetidylpeptidase IV the latter signaling seems likely. Supporting this suggestion, in contrast to the ability of GLP-1 for activating receptors in the brain stem to activate vagal motor neurons sending impulses back to the pancreas and gastrointestinal tract, so far GIP receptors could not be identified in this brain region ( 137 , 138 ). Therefore, GIP treatment may not be successful in facilitating body weight decrease as it was reported for brain-stem mediated appetite reduction with GLP-1 treatment. Indeed, the impact of GIP on central appetite regulation is controversial ( 139 , 140 ). In the brain, the GIP receptor is mainly expressed in the cerebral cortex, hippocampus, olfactory bulb, lateral septal nucleus, subiculum inferior colliculus and inferior olive ( 137 , 138 ). In the dentate gyrus of the hippocampus additionally to GIP receptor expression, GIP protein expression has

been reported ( 134 ). Interestingly, hippocampal gene expression and action of GIP varied strongly in parallel with cell proliferation rates in the adult rat dentate gyrus. In addition, i.c.v injection of GIP increased synaptic plasticity in the hippocampus and even protected synapses from the detrimental effects of beta-amyloid fragments involved in Alzheimers Disease development ( 141 ). This implies that local and paracrine action of GIP in the hippocampus may contribute to appropriate hippocampal cell genesis and spatial learning performance ( 134 ).

In the periphery, the GIP receptor is expressed in various tissues, including the pancreas, white adipose tissue, heart and adrenal cortex ( 135 , 137 ). The most prominent phenotype of GIP receptor deficient mice is their resistance against diet-induced obesity that is obviously mediated by increased energy expenditure and increased fatty acid oxidation ( 135 ). The adipogenic action of GIP is obviously due to its ability of stimulating the synthesis and secretion of lipoprotein lipase in white adipocytes ( 135 , 142 ). In humans, GIP infusion in the presence of hyperinsulinemia also increased glucose uptake and free fatty acid re-esterification, thus resulting in increased TAG deposition in abdominal subcutaneous adipose tissue ( 143 ).

Taken together, the GIP characteristics of maintaining appropriate hippocampal cell genesis, inducing glucose-dependent insulin secretion, and increasing adipose tissue lipoprotein function indicate that naturally GIP may contribute to spatial learning important for foraging and to efficient storage of ingested nutrients.

*Ghrelin* , identified in 1999, has received enormous research attention as a multifunctional hormone connecting several organs and physiological functions (e.g. food intake, stomach emptying, locomotor activity, energy expenditure, nutrient partitioning, lipid-and glucose metabolism). Ghrelin is released from the stomach in anticipation of a meal, and is one of the only known peripheral signals that stimulate food intake rather than causing satiety. Chronic administration of ghrelin results in obesity in rodents ( 144 ), and acutely administered it will cause animals and humans to consume larger meals than normal ( 145-147 ). As would be expected, most obese humans have low levels of circulating ghrelin, whereas levels are elevated in patients with anorexia nervosa ( 148 ). Centrally, small amounts of ghrelin are synthesized in a subset of neurons in the ventral hypothalamus although it has proven difficult to consistently define this population by immunocytochemical and other techniques ( 149 ). The ghrelin receptor (growth hormone secretagogue receptor, GHS-R) is expressed within hypothalamic NPY and AgRP neurons in the ARC, making them a target for ghrelin and ghrelin's actions functionally depended on NPY/AgRP neurons ( 25 , 58 ). NPY/AgRP neurons are activated by centrally administered ghrelin which in turn potentiates the inverse agonist effect of AgRP on hypothalamic melanocortin receptors, resulting in a reduction of α-and ß-MSH release by POMC neurons. In addition, ghrelin mediated activation of NPY/AgRP neurons leads to GABA induced inhibition of POMC neuron activity ( 77 ). This creates an attenuation of hypothalamic satiety tone ( 58 ) by enhancing the firing rate of NPY neurons while diminishing POMC neuron firing. Apart from its actions on neurons in the ARC, ghrelin administration into other areas of the brain (i.e. PVN, LHA, ventral tegmental area (VTA), dorsal vagal complex) also stimulates a positive energy balance ( 150-154 ). One of the most prominent characteristics of ghrelin is its adipogenic potency, making it an attractive target for the treatment of obesity. However, enthusiasm for ghrelin antagonists as anti-obesity drugs is combined with skepticism

since ghrelin levels in obese humans are already low ( 155 , 156 ). It was shown in animal studies that the effect of decreased ghrelin action depends on the age, gender and diet of the animals and does not always result in a lean and hypophagic phenotype ( 157-160 ). Although studies using ghrelin antagonists and vaccinations against ghrelin have shown significant effects on energy balance in lean and obese adult rats, the effects of a hypothetical ghrelin antagonist in humans is unclear ( 156 , 161-163 ). Studies in ob-mice with an additional deletion of ghrelin, i.e. a double knockout for leptin and ghrelin, showed these animals had improved glucose tolerance and insulin secretion and action compared to regular *ob/ob* mice ( 164 ). This role of ghrelin in the control of glucose homeostasis suggests additional therapeutic potential for the treatment of type II diabetes.

*Peptide YY (PYY)* is released from the L cells of the distal digestive tract and has been shown to act as a satiety signal ( 165-167 ). PYY belongs to the pancreatic polypeptide family, which further includes pancreatic polypeptide (PP) and NPY. The peptide is present in two circulating forms, PYY1-36 and PYY3-36, the latter is the main circulating form of PYY in postprandial human plasma and able to cross the blood-brain-barrier freely by non-saturable mechanisms ( 168 , 169 ). Both forms of PYY bind to the Y2 isoform of the NPY receptor ( 170 , 171 ). The effects of PYY3-36 on food intake in rodents and humans have been controversial with several groups reporting conflicting results. Experiments where PYY3-36 was given peripherally showed a reduction in food intake in rodents and humans and decreased body weight gain in rodents, suggesting a potential anti-obesity therapeutic action ( 167 ). Furthermore it was reported that PYY levels are significantly lower in obese patients. However, other groups were unable to reproduce any anorexigenic or weight reducing effects of PYY3-36 in rodents, and instead confirmed normal PYY levels in established obesity ( 171 , 172 ).

*Amylin* is a peptide hormone secreted by pancreatic □-cells together with insulin secretion during meals. Amylin has been shown to inhibit gastric emptying and systemic and central administrations cause a dose-dependent reduction of meal size ( 173-176 ). A combination treatment of amylin and leptin elicited greater inhibition of food intake and body weight loss in obese rats, than predicted by the sum of mono-therapy conditions and peripheral administration of amylin restores leptin sensitivity in rats, crucial in the treatment of leptin resistance in obesity ( 177 ). These observations highlight the potential of combination therapies for obesity.

Nutrient-related signals including *glucose and free fatty acids* have received intense attention and research particularly in the last five years. Free fatty acids have been shown to exert insulin-like effects in key brain areas, including the ARC. One possible route of action is via the intracellular accumulation of long-chain fatty acyl-Coenzyme A, which is thought to play a critical role in energy homeostasis given that selectively reduced levels of this enzyme in the ARC result in obesity ( 178-180 ).

*Glucose* sensing, for instance, is maintained by 1) glucose excited (GE) neurons that increase activity and 2) glucose inhibited (GI) neurons that decrease activity with rising glucose levels. These neurons represent distinct cell populations. However, they often co-exist in the arcuate nucleus, where POMC expressing neuron population overlap with GE neurons and NPY expressing neuron populations overlap with GI neurons ( 5 ). In addition, MCH neurons in the lateral hypothalamus have been identified as being GE neurons and orexin neurons in the same

nucleus were suggested to be GI neurons ( 5 ). Intrahypothalamic glucose administration decreases food intake and inhibits hepatic glucose production, suggesting that glucose sensing neurons may regulate appetite and blood glucose concentrations ( 5 ). Intriguingly, the activity of glucose sensing neurons is regulated not only by glucose concentrations, but also be the presence of hormones like leptin and insulin as well as by the release of neuropeptides (NPY, α-MSH or orexin) ( 5 ). Therefore, glucose may not be a primary regulator of food intake but part of a signal machinery that in its sum regulates food intake and digestion via changes in the membrane potential of neurons ( 181 ).

In addition to glucose, recent studies have shown that sensing of *long-chain fatty acids (LCFA)* in the hypothalamus contributes to regulation of food intake ( 182 ). LCFA-CoAs have been recognized as a key signal to hypothalamic neurons in response to increased availability of fatty acids in states of nutrient surfeit ( 182 ) leading to inhibition of food intake and hepatic glucose production (168). This effect was mediated by NPY/AgRP neurons. In addition, the precursor of intracellularly synthetized LCFAs, Malonyl-CoA, which arises from glycolysis, also plays a critical role by inhibition of carnitine palmitoyltransferase (CPT-I), thereby preventing LCFAs from being oxidized in the mitochondria. Neuronal malonyl-CoA levels in the hypothalamus may also increase by centrally and peripherally administered fatty acid synthase (FAS) inhibitors (e.g. C57) ( 183 ). Furthermore, inhibition of AMP-activated protein kinase (AMPK) leads to dis-inhibition of acetyl-CoA carboxylase (ACC) and therefore to potentiated production of malonyl-CoA, which in turn results again into increased LCFA levels. Intriguingly, in the hypothalamus, AMPK is also inhibited by anorexic signals like leptin, insulin and glucose presumably resulting in increased neuronal LCFA concentration to decrease food intake. Therefore it was proposed that metabolic and hormonal signals may converge at the point of FA sensing inside the hypothalamus to regulate food intake ( 182 ). Supportive of this hypothesis, FAS immunoreactiviy co-localizes with NPY immunoreactivity in the hypothalamus, which is a common player in mediation of leptin, insulin and ghrelin induced effects on food intake ( 182 ). Furthermore, treatment with FAS inhibitors led to decreased food intake associated with decreased expression of NPY/AgRPand elevated expression of CART/POMC in ARC neurons, and this effect was malonyl-CoA dependent ( 184 ).

Very recent findings also implicate *amino acids* , especially the branched-chain amino acid leucine, in the hypothalamic regulation of food intake. Intracebroventricularly administered leucine decreased food intake and mRNA content of AgRP in the hypothalamus.

- While there are several indications of interplay between metabolic, hormonal and neuropetidergic signals to regulate food intake and energy balance, the molecular underpinnings of this integration are far away from being elucidated yet. It appears that the information carried by afferent signals converge in several common intracellular pathways involving especially certain fuel sensors like AMPK and mTOR ( 185 ). AMPK is activated in states of negative energy balance, when intracellular ATP:AMP ratio is low. In contrast, mTOR is activated in states of positive energy balance, when intracellular ATP:AMP ratio is high ( 185 ). In the ARC, the catalytic subunits of AMPK co-localize with NPY, and AMPK activation stimulated NPY expression ( 185 ) and thus may increase food intake. Activation of mTOR, however, may decrease food intake. Intriguingly, the phosphorylated form of mTOR also highly localizes to the NPY/AgRP

and POMC neurons of the ARC. So far, changes in mTOR activity have been reported in relation to hypothalamic leucine sensing ( 185 ). In addition, leptin as well as insulin activate PI3K and this activation is obviously necessary for appropriate reduction in food intake ( 21 ). The convergence of the two anorectic hormones in the activation of PI3K might be the basis for their congeneric action in the hypothalamus to decrease food intake. If this may occur concomitantly within the same population of neurons still needs to be elucidated ( 21 ). For further information on the convergence of hormonal and nutrient sensing in intracellular signaling inside the hypothalamus see these elaborate reviews ( 21 , 185 , 186 ).

## What has the hindbrain got to do with it?

The brainstem is crucial for the regulation of energy balance. It contains neurons involved in the autonomic control of energy balance as well as motor pattern generators which orchestrate descending command signals from the forebrain that are then translated into coherent movements. Particularly in the last 15 years considerable progress has been made in demonstrating the role of the brainstem in both ingestive behavior and energy balance, and it is now considered an integral part of the overall feeding network.

Recently evolving studies indicate that both sensory and integrative functions are distributed across the basal forebrain as well as the caudal brainstem. A strong presence of leptin and insulin receptors, glucose-sensing mechanisms, and neuropeptide mediators in the brainstem suggest its pivotal role in coordinating energy balance regulation. In addition, a physiological relevance is suggested by the demonstration that similar effects can be triggered independently by stimulation of respective forebrain and brainstem subpopulations of the same receptors ( 18 ). Supporting this statement, animal studies have shown that administration of an MC4-R agonist (Melanotan II, MTII) either into the lateral or into the fourth ventricle decreased food intake and body weight and increased expression of UCP1 in brown adipose tissue and body core temperature ( 187 , 188 ), whereas injection of an antagonist (synthetic melanocortin analogue, SHU9119) showed an increase in food intake and body weight ( 188 , 189 ). CART-expressing neurons are also located in the area postrema (AP) and parts of the NTS including the dorsal vagal complex (DVC) ( 190 , 191 ), but do not co-express POMC as observed in the ARC. In rodents, injection of CART peptide into the fourth ventricle results in suppressed food intake ( 191-193 ), which was similarly reported for lateral ventricle injections hitting the hypothalamus ( 194 ). In addition, NPY-expressing neurons are located in the NTS and fourth ventricular injection of NPY has been shown to increase food intake in sated rats.

Furthermore, decerebrated (transection at the pontine level leaving the brain stem intact) and neurologically intact rats show similar responses to taste stimuli or starvation and are similarly sensitive to inhibitory feedback from the gastrointestinal tract, indicating that the caudal brainstem is sufficient to mediate ingestive responses. It was shown that the brainstem can perform its functions in neural isolation of the forebrain, being able to mediate ingestive responses to a range of afferent visceral stimuli i.e. ingestive responses to insulin-induced hypoglycaemia, normal satiation, and suppression of intraoral sucrose intake by CCK ( 35 , 195-197 ).

However, experiments in decerebrated rats have shown that a functional forebrain-caudal brainstem connection is essential for the occurrence of catabolic leptin effects, while leptin appears to act anabolic, when this connection is lost ( 198 ). Also hypothalamic-neuroendocrine responses to fasting depend on ascending pathways from the brainstem. This suggests that control mechanisms endemic to the hypothalamus and brainstem drive their unique effector systems on the basis of local interoceptive and visceral (in the case of the brainstem) afferent inputs and that a set of uni- and bidirectional interactions between these structures coordinates adaptive neuroendocrine, autonomic, and behavioral responses to changes in metabolic status ( 199 ).

Food intake initiates the release of vagal-mediated gastrointestinal hormones (i.e. CCK, GLP-1) into the circulation. These gastrointestinal hormones then in turn affect neuronal brainstem function in the area postrema and in the NTS, as these structures have a weak or absent blood-brain-barrier ( 200 , 201 ) and express respective receptors (e.g. CCK and GLP-1 receptors)( 202-205 ). These areas then integrate this information with taste information from the oral cavity. As described earlier, the gut peptide PYY has been shown to have anorexigenic effects when acting in the hypothalamus, however when PYY is infused into the fourth ventricle it has been shown to potently increase food intake. These discrepant effects might be due to the varying forms of either full-length PYY or the circulating PYY3-36 and their differing affinity for either the Y2 or the Y1 receptors, with Y1 receptors being responsible for increasing food intake. Similarly, ghrelin receptors are located in the AP and NTS and direct injection of ghrelin to the fourth ventricle has been shown to stimulate food intake. It should also be appreciated that sensory signals pertaining mainly to the short term reaction to food intake and energy fluxes are relayed either from the gut to the brainstem (i.e. CCK) ( 121 , 206 ) or directly to the mediobasal hypothalamus (i.e. ghrelin) as discussed in previous paragraphs ( 207 ).

Recent data supporting the involvement of the brainstem have shown that interoceptors (sensory receptors that detect changes in certain endogenously derived signals) are more widely distributed throughout the brain than originally thought. Interoceptors found in the brainstem include leptin and insulin receptors, ghrelin, glucose-sensing mechanisms and neuropeptide (i.e. α-MSH, CRH) mediators. It has now been accepted that the long-form leptin receptor (Ob-Rb) is present in several caudal brainstem areas including all three divisions of the dorsal vagal complex and parabrachial nucleus that are known to be implicated in the control of food intake. Animal studies have shown that leptin injections into the fourth ventricle decreased food intake and suppressed body weight. Insulin receptors have been localized in the AP and NTS ( 208 , 209 ). In addition, a small group of neurons in the caudal part of the NTS express POMC, the only brain area apart from the ARC where POMC expression is found (s. also under “The CNS melanocortin system”).

## How the hypothalamus and the brainstem communicate to regulate energy balance

Communication between the forebrain and the brainstem, i.e. communication between adiposity signals (i.e. leptin and insulin) and satiety signals (i.e. CCK, GLP-1) is essential to adjust sensing and integration of satiety signals according to the status and requirements of body

energy stores. This is supported by data showing that both leptin and insulin enhance the satiating effect of CCK ( 210 , 211 ). One possible explanation for this interaction is the ability of central effector pathways to influence the response of NTS neurons to input from vagal afferents that convey satiety-related stimuli. The fact that leptin potentiates the effect of CCK in activating neurons in the NTS clearly shows that signals involved in the long-term regulation of energy balance do change the response of NTS neurons to specific satiety related inputs.. The stimulating effect of leptin could be due to the fact that leptin receptors are expressed by a subset of nodose ganglion neurons (sensory cell bodies of vagus nerve) and modulate CCK-sensitive vagal afferent fibres innervating the gastrointestinal tract ( 212-214 ). In addition, the neuronal substrates responding to central effector peptides (i.e. α-MSH, CART, NPY) involved in energy homeostasis are expressed in neurons located within the NTS. E.g., given the moderate to high expression level of MC4-R in the caudal medulla, NTS, and even higher expression in the dorsal vagal complex, POMC neurons in the NTS are likely to provide some of the ligand for the MC4-R in the hindbrain.

Further supporting data for the interaction between the fore-and hindbrain is the fact that NTS neurons have reciprocal interconnections with forebrain areas such as the PVN, highlighting that the integration of satiety and adiposity signals involves several brain areas and is not restricted to the mediobasal hypothalamus.

Projections to and from the brainstem have been identified using various retrograde and anterograde tracing techniques. In short, the DVC receives direct projections from the hypothalamus (mainly PVN and LHA), amygdala and prefrontal cortex (i.e. agranular insular cortex) ( 215-219 ). In further support of interactions between the forebrain and the brainstem, MCH and orexin neurons located in the LHA have direct projections to the brainstem and receptors to both peptides have been shown in the NTS and dorsal motor nucleus ( 87 , 88 , 220-229 ).

In summary, the brainstem holds an expansive array of neurons and circuits that are directly involved in ingestion, digestion and absorption of food. It is currently assumed that the brainstem in isolation would not be able to respond appropriately to a long-term homeostatic challenge but is effective in responding to short-term gastrointestinal signals.

## Stepping outside the homeostatic square: extra-hypothalamic centers involved in reward, hedonism and cognition **contribute to body weight regulation**

There are other brain areas besides the hypothalamus and brainstem that play important roles in regulating energy balance. For example, it is critical that appropriate motor function is exerted in order to both gather food as well as to appropriately process ingested nutrients.

The homeostatic regulation of energy balance is remarkably powerful in defending the lower limits of adiposity (bias towards weight gain, ( 23 ), and weak in curbing appetite to prevent weight gain in the current world of abundance of highly palatable high energy foods. Non-

metabolic sensory factors that lead the homeostatic system to become unbalanced and contribute to overeating and obesity include food palatability, sensory-specific satiety, fixed meal times, food salience and portion size, energy density of food, consumption rate, stress, social environment and energy output/exercise ( 230 , 231 ). Palatability and pleasantness of food are arguably the most powerful determinants in regulating motivation to eat.

As discussed in the previous section, hardwired homeostatic brain mechanisms contribute to the control of appetite, however, a number of sensory and environmental factors of the “external world” contribute to over-stimulation of the sensory systems, producing sensory reward signals that sometimes overwhelm satiety signals. While satiety signals (gut-hormones i.e. CCK) presumably represent a physiological constant of meal termination regulation over the last centuries, changes on the sensory side (produced by taste, smell, texture, appearance of food, availability) have increased and changed dramatically during the last decades. In other words, satiety signals are simply being overridden by cognitive and reward stimuli, contributing to the increasing incidence of obesity. This inequality pushes the energy balance equation towards weight gain and eventually obesity. Brain areas that were evolutionarily occupied with procuring and ingesting food not only involved the hypothalamus but also corticolimbic structures such as the amygdala, nucleus accumbens and prefrontal cortex. These structures are more involved in the rewarding aspect of food ingestion and are simply overwhelmed in today’s society which has both an abundance of food and food cues and freedom from famine. It is now increasingly recognized that humans and animals are unlikely to be driven to obesity by failure of homeostatic mechanisms alone and the role of non-homeostatic reward/hedonistic pathways and the way in which they interact with cognitive and homeostatic pathways has moved closer to center stage ( 232 , 233 ).

Decades of research on cortical and basal ganglia function have led to the development of models of higher-order (corticolimbic) executive control of voluntary motor behavior, including that of feeding where critical aspects of motivational feeding are controlled ( 234 ). The hedonic impact of preferred foods is now thought to be mediated by networks linking the amygdala, prefrontal cortex and ventral striatum (including the core and shell of the nucleus accumbens).

The increase in obesity worldwide supports the idea that, particularly in humans, the initiation of a meal often starts as a purely cognitive/executive decision from the cortex in the absence of any depletion signal. Thus, even in the presence of satiation and repleted energy stores, it is possible for the cortex and limbic system to overpower the hypothalamus into an ingestive mode. The brain areas involved in the hedonistic and reward control of energy balance include the nucleus accumbens, which provides an interface with its afferent and efferent connections between motivational aspects and behavioral motor responses ( 235 , 236 ), the ventral tegmental area (VTA) in the midbrain, the orbitofrontal cortex, the amygdala and the thalamus which acts as an integrator for homeostatic and hedonistic signals ( 237 ).

The mesolimbic pathways (dopaminergic projections between the VTA and the nucleus accumbens) subserve reward based feeding ( 236 , 238 , 239 ) but how they are incorporated into the greater scheme of the CNS control of energy balance remains to be elucidated. Dopamine has been shown to potently augment the drive to obtain a rewarding stimulus. One mechanism that may promote consumption of a palatable food through activation of the VTA-

nucleus accumbens pathway involves the projections to the LHA which is known to contain orexigenic peptides. Various tracer studies have shown projections from mainly the shell of the nucleus accumbens to the LHA/PeF ( 240-243 ). These pathways support the anatomical and functional view of the LHA as an integrative point for not only homeostatic but also reward-related inputs that can collectively stimulate feeding behavior and might be an important pathway for the “cognitive” brain to override homeostatic regulation. Supporting data show that orexin neurons in the LHA and NPY neurons in the ARC were activated while POMC/CART neurons were deactivated by manipulation of the nucleus accumbens with muscimol (selective GABA agonist) ( 244 ). Additionally, current data suggests that also the adiposity signals insulin and leptin play a role in modulating reward function. This hypothesis is supported by the expression of both insulin and leptin receptors throughout the limbic forebrain including the nucleus accumbens and VTA ( 245-248 ). Emerging evidence now supports the idea that the VTA is sensitive to insulin, leptin and also ghrelin and that these signals can modulate the activity of dopaminergic neurons within the VTA ( 248-251 ). One possible mechanism suggests that low circulating levels of insulin and leptin, indicating energy restriction, is able to increase sensitivity of reward circuits ( 252 , 253 ). This hypothesis is supported by data from animal studies where the administration of insulin or leptin diminishes sucrose preference which is a measure for food reward ( 253 ).

The cortical loci of the motivation to eat have proven difficult to isolate but evidence supporting the involvement of cortical regions including the prefrontal/orbitofrontal, anterior cingulate and insular cortex has been derived from both imaging ( 254-258 ) and electrophysiological studies ( 259-261 ). Recent imaging studies have also highlighted the anatomical correlates of mesolimbic reward pathways ( 262 ) and their underpinning of the phenomena of “wanting” and “liking” as proposed by Berridge and colleagues in relation to food intake ( 263 ). Interestingly, the adipocyte derived hormone leptin and the gut derived hormones ghrelin and PYY are likely to act locally to influence the activity of this pathway.

“Primary taste neurons” are located in the insular cortex that are responsive to sweet, salt, bitter, sour and umami taste. They are supplied by NTS neurons by way of the thalamus. These neurons provide representations of food in the mouth independently of hunger and thus of reward value and pleasantness. These cells project in turn to “secondary taste neurons” in the orbitofrontal cortex that integrate taste information with olfactory and visual inputs. The orbitofrontal cortex is responsible for forming learned associations and is a crucial site for interactions between sensory inputs produced by food and hunger/satiety signals. This system determines how pleasant a food is and whether we have an appetite for it or; in other words, the reward value is explicitly represented in the area where satiety signals modulate the response of the taste and flavor neurons. The orbitofrontal cortex is known to be a site where pleasantness and palatability of food are represented. It has connections to adjoining areas, including the cingulate cortex and nucleus accumbens, through which it can drive eating behavior.

In light of the above, the challenge is to identify and understand the nature of the integration of three key pathways: 1) the homeostatic regulation directed primarily from the ARC and brain stem, 2) the hedonistic/motivational regulation seated in mesolimbic pathways and 3) the afferent pathways that convey this information to “higher” cortical brain regions.

Food intake is thus controlled by building a multimodal representation of the sensory properties of food in the orbitofrontal cortex, which is then gated by satiety signals to produce a construct of the pleasantness and reward value of food which subsequently drives food intake. Therefore, body weight regulation not only depends on homeostatic signals emerging from nutrient supply and energy stores but it is also influenced by hedonistic and reward stimuli that may shift energy balance toward food intakes higher than energy expended leading in the long-term to body weight gain and possibly obesity. This implies that pharmacologic treatment of obesity may not only include medication targeting homeostatic signals but also compounds with combined targeting of homeostatic and hedonistic/reward signals.

# SUMMARY

This chapter attempts to provide an overview of the known physiological processes that occur in the brain to integrate and analyze a large variety of afferent signals reflecting energy requirements and that respond with the appropriate compensatory changes in appetite, metabolism and behavior. Immediate conditions instantly induce hormonal and neuronal messages from the periphery that communicate to distinct areas of the brain the need for regulatory changes in order to maintain energy balance. It is within these brain areas that not only sensations such as hunger and satiety are created, but also outgoing impulses for food seeking behavior, changes in locomotor activity or appropriate modulations of peripheral metabolic drive are triggered. The localization and precise action of these brain centers, as well as the exact mapping of their interactive signal transduction pathways, remain largely unknown despite great scientific progress in this field during the last two decade. A finely tuned balance of action potentials, synaptic neurotransmitters, feedback loops and neuropeptide expression levels between regulatory centers in the brainstem, hypothalamic nuclei, basal ganglia, nucleus accumbens and even the cortex underlies the constant adjustments that take place. The redundant multiplicity of factors governing energy balance, that have been generated due to the evolutionary need to ensure sufficient caloric intake, is regarded as one reason for the continued failure to generate an effective pharmacotherapy for obesity.

# Neuropeptides in hypothalamic circuits involved in metabolism regulation

The past decades have provided overwhelming evidence that the principle signaling modality within brain centers of energy balance regulation is via chemical synapses. As eluded earlier, chemical neurotransmission occurs by the release of neurotransmitters and neuropeptides from synaptic vesicles of axon terminals onto their respective receptors located at the postsynaptic membrane or membranes of adjacent axon terminals. Studies indicate that the central regulation of feeding behavior and energy expenditure relies on the appropriate interaction between particular neuropeptides. In this regard, those key hypothalamic neuropeptide systems that have been most closely associated with metabolism regulation are summarized below. However, some other peptides that are involved to a lesser extent will not be discussed. A detailed and thorough description of these peptides and their functions can be found in elaborate reviews ( 1 , 19 , 22-33 , 199 , 264 , 265 ).

## Orexigenic neuropeptides and brain derived factors

### Neuropeptide Y (NPY)

NPY, a 36-amino acid peptide is one of the most abundant and widely distributed neuropeptides within the nervous system and is one of the most potent stimulators of feeding. NPY administered repeatedly into the hypothalamus induces obesity accompanied by hyperphagia, decreased thermogenesis in brown adipose tissue, hyperinsulinemia, hypercorticosteronemia, reduced plasma testosterone levels and insulin resistance in skeletal tissues. The levels of NPY mRNA in the arcuate nucleus respond to changes in energy status. For example, they are increased during fasting and chronically up-regulated in many rodent obesity syndromes, e.g., in the *ob/ob* mouse. At least 5 distinct receptors (Y1, Y2, Y4, Y5 & Y6), all belonging to the G-protein coupled receptor superfamily, mediate the actions of NPY ( 19 , 266 ).

### Agouti-related protein (AgRP)

AgRP is a neuropeptide produced in the arcuate nucleus. AgRP represents an endogenous antagonist (inverse agonist) of MC3/4R that decreases receptor activity and induces positive energy balance by increasing food intake and decreasing energy expenditure.

### Pro-opiomelanocortin (POMC)

POMC is a large precursor peptide that is cleaved within neurons to several specific peptides. These includemelanocyte stimulating hormone (α-, β-, γ-MSH), ß-Endorphin and adrenocorticotropin homone (ACTH) with opposing effects on energy balance. Within the hypothalamus, POMC neurons, localized exclusively in the arcuate nucleus, innervate the paraventricular nucleus, dorsomedical nucleus, and other areas of the hypothalamus. ß-Endorphin increased food intake.

### Melanin-concentrating hormone (MCH)

A population of neurons in the zona incerta and lateral hypothalamus (LH) produce the 19-amino acid peptide, melanin-concentrating hormone, and project to several hypothalamic, limbic and cortical areas. Studies revealed several parallels between MCH and NPY systems in the hypothalamus. MCH augmented ongoing feeding; fasting stimulated MCH gene expression in the hypothalamus, and MCH mRNA was elevated in genetically obese *ob/ob* mice. MCH also stimulated the hypothalamo-pituitary-adrenal axis. Orexigenic effects of MCH in rats have also been observed, but relative to NPY, MCH-induced feeding seems less impressive and of shorter duration. Further, despite the acute stimulatory effects of MCH, cumulative 24-h intake was unaffected, and repeated daily injections stimulated food intake for a few days without changing the body weight. Interestingly, small lesions in the VMN that stimulated MCH gene expression in the hypothalamus failed to evoke hyperphagia ( 19 , 267 ).

### Hypocretins/orexins (A,B)

Hypocretins 1 and 2 have been localized in clusters in the dorsal and lateral and perifornical hypothalamic area. Intracerebroventricular injections of hypocretin 1 (orexin A) and hypocretin 2 (orexin B) stimulated feeding in a dose-related fashion with orexin A significantly more effective than orexin B, possibly due to activation of both orexin A and B receptor subtypes. Orexin was found to be less effective than NPY in stimulating food intake and, as with NPY and MCH, fasting up-regulated orexin gene expression in the hypothalamus. A comparative evaluation of the potency of orexins with other orexigenic signals examined thus far indicates that higher doses of orexins (given by intracerebroventricular injection) than of other substances (galanin, MCH, or gamma-aminobutyric acid) are needed to elicit significant stimulation of feeding.

### Hypothalamic ghrelin

A uniquely distributed hypothalamic group of mostly bipolar neurons has been identified as producing small amounts of ghrelin. These neurons are not co-localized with any known centrally expressed hormone or neuropeptide, but, intriguingly, they do project directly to several previously identified hypothalamic appetite control centers. Furthermore, ghrelin receptor expression and binding is localized in multiple hypothalamic areas that neighbor NPY, AGRP, POMC, GABA and other neuropeptides and neurotransmitters substantially involved in appetite control. Ghrelin expression is found in neurons situated close to, but not connected with, the previously mentioned neuropeptides. These neuroanatomical findings, complemented by electrophysiology studies, provided evidence for the existence of a central circuit regulating appetite involving ghrelin as a key-modulator ( 149 ).

### Brain-specific homeobox transcription factor (Bsx)

This hypothalamic factor plays an essential role in the generation of hyperphagic responses. It increases NPY and AgRP gene expression and ghrelin stimulated food intake ( 268 ). Bsx expression in the arcuate nucleus was increased following ghrelin administration ( 269 ).

## Anorexigenic neuropeptides and brain-derived factors

### Melanocyte stimulating hormone ( a -MSH)

a-MSH is a melanocortins that is cleaved from the pro-opiomelanocortin (POMC) precursor molecule. It acts as an endogenous agonist of the MC3-R and MC4-R, the two melanocortin receptor subtypes that are thought to be important in the regulation of food intake.The melanocortin system is thought to be one of the most significant pathways involved in the regulation of food intake, with mutations within the system found in approximately 2% of the cases of genetic obesity in humans.

The melanocortin system has an endogenous antagonist, agouti-related protein (AgRP), co-expressed in the same neuronal population as NPY.

### Corticotropin-releasing hormone (CRH)

CRH has opposite effects to NPY. It inhibits feeding and stimulates metabolic rate when injected intracerebroventricularly in animals. Multiple subpopulations of CRH-producing neurons, CRH-immunoreactive terminals, and high-affinity binding sites have been localized in various regions in the brain. Microinjection studies revealed that the sites of anorectic action of CRH lies within the PVN, possibly mediated by CRH R1 or CRH R2 receptor types. The fact that intraventricular injection or microinjection of CRH into the PVN, and not elsewhere in the hypothalamus, inhibited NPY-induced feeding further strengthened the notion that CRH, if released locally in the PVN, may tonically restrain the action of endogenous orexigenic signals( 270 ). Urocortin, a recently described member of the CRH family with 45% sequence homology to CRH, has been shown to be more potent than CRH in suppressing both the fasting induced and nocturnal feeding. Reduction of nocturnal feeding by urocortin was found to be due to a reduction in meal size and not frequency of meal bouts. This observation questions the physiological significance of this anorexic peptide in nocturnal feeding marked by a robust increase in both meal size and frequency. The topographies of urocortin and CRH-expressing cells in the rat brain are quite different and interesting. Urocortin-expressing cells are found in the Edinger-Westphal nucleus, the lateral superior olive, the LH and supraoptic nucleus (SON), but not in the PVN. The higher anorectic potency of urocortin has been attributed to a relatively higher affinity of urocortin for CRH R2 and its splice variant CRH R2a. Although urocortin immunoreactive nerve fibers innervate the lateral septum, VMH, and medial amygdaloid nucleus, urocortin microinjections into the VMN, but not into the PVN, inhibited feeding ( 270 ).

**Neurotensin**

Neurotensin (NT), isolated and characterized in the early 1970s, inhibits spontaneous and norepinephrine-induced feeding in rats, and there is evidence that NT and dopamine act synergistically to inhibit feeding. The neuroanatomical mapping of NT pathways in the rat hypothalamus is consistent with the existence of anorexigenic pathways. Within the hypothalamus, NT-like immunopositive neurons exist in several distinct nuclei. Notable among these are subsets of NT-producing neurons in the ARC, PVN, and DMN. In addition, these and neighboring sites are richly innervated by NT-immunopositive fibers. Interestingly, recent studies showed that a subset of NT-positive neurons in the DMN project to both the parvicellular and magnocellular PVN, sites in which microinjection of NT inhibited spontaneous feeding. In addition, consistent with a reciprocal interaction between NPY and NT underlying hyperphagia in rodents, it has been reported that *ob/ob* mice exhibit decreased hypothalamic NT mRNA and peptide levels in association with enhanced NPY levels and gene expression ( 271 ).

**Glucagon-like peptide-1**

Glucagon-like peptide-1 (GLP-1) (7-36) amide is processed from proglucagon in intestinal L cells, and it is considered to be a hormone related to the glucagon/secretin family of peptides. Like several other gastrointestinal peptides, GLP-1 has been found in various forebrain sites and in hypothalamic sites that correspond with GLP-1-binding sites in the ARC and PVN. Extensive hypothalamic innervation by GLP-1-immunoreactive fibers apparently emanates from a single population of non-catecholamine-producing neurons in the caudal portion of the nucleus of the Solitary tract. Intraventricular administration of GLP-1 inhibited food intake in fasted rats, a response blocked by the concurrent administration of exendin (9-39), a

GLP-1-receptor antagonist. A physiological role of GLP-1 as an anorectic or satiety factor was suggested by the observations that exendin stimulated feeding in satiated rats during the lights-on period, and daily injections of exendin augmented food intake and body weight. Evidence suggests that one of the sites of GLP-1 action may be the PVN where GLP-1-immunoreactive fibers terminate and where exendin blocked GLP-1-induced activation of c-FOS. The anorectic effects of GLP-1 may be mediated through NPY signaling because GLP-1 inhibited, and exendin (9-39) augmented, NPY-induced feeding. Suppression of feeding by GLP-1 likely involves inhibition of postsynaptic signaling initiated by NPY in the PVN and not by suppression of NPY synthesis in the ARC. The fact that GLP-1 may be an endogenous anorectic signal was also indicated by the report that attenuation of feeding by GLP-1 was not due to conditioned taste aversion ( 272 ).

**Cocaine and amphetamine-regulated transcript (CART)**

Cocaine and amphetamine-regulated transcript is localized in the hypothalamus and shown to be distributed in feeding-related sites in the hypothalamus. Intracerebroventricular administration of CART inhibits normal and starvation-induced feeding. CART administration markedly inhibits the NPY-induced feeding response.

**Nesfatin-1**

Nesfatin-1 represents an amino-terminal fragment derived from NEFA/nucleobindin2 (NUCB2) and is expressed in several nuclei of the hypothalamus that are involved in the regulation of energy balance ( 273 ). I.c.v. injection of nesfatin-1 decreases food intake in a dose-dependent manner. Central injection of *a* -MSH elevates NUCB2 gene expression in the PVN, and satiety by nesfatin-1 is abolished by an antagonist of the MC3/4R. It was therefore suggested that nesfatin-1 is a satiety molecule associated with melanocortin signaling in the hypothalamus ( 273 ).It was demonstrated that nesfatin-1 neurons in the PVN and supraoptic nucleus (SON) overlap extensively with oxytocin and vasopressin neurons and to a lesser extend with CRH and TRH neurons ( 274 ). Refeeding selectively activates nesfatin-1 neurons in the PVN and SON and increases nesfatin-1 expression in the SON. It was hypothesized that nesfatin-1 neurons in the PVN and SON may play a role in the postprandial regulation of feeding and energy homeostasis ( 274 ).

**Brain-derived neurotrophic factor (BDNF)**

BDNF is one of four structurally related proteins that belong to the neurotrophin family. Neurotrophins generally promote neuronal survival and development within the peripheral nervous system. BDNF and its tyrosine kinase receptor, TrkB, are expressed in several hypothalamic nuclei associated with satiety and locomotor activity and mice heterozygous for a targeted disruption of the BDNF gene are obese and hyperactive( 275 ). The synthesis of BDNF in the VMH and/or DMH was shown to be required for the suppression of appetite ( 276 ). BDNF expression in the VMH is regulated by nutritional state and by MC4R signaling( 277 ). Obviously MC4R signaling controls BDNF expression in the VMH and it was hypothesized that BDNF is an important effector through which MC4R signaling controls energy balance ( 277 ).

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