Brain Regulation of Feeding and Energy Homeostasis

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Abstract

In the past decades, it has become clear that the brain plays a key role in the control of feeding and energy homeostasis. These are complex systems that require the integration of diverse physiological components, from sensing energy demands and storage to behavioral responses, motor function, and reflex adjustments. Studies in different organisms from worms to flies and rodents to humans have identified key molecular pathways, conserved genes, and neural circuits crucial for the understanding of the control of distinct components of energy homeostasis. Among them, the brain plays a fundamental role in food intake (e.g., meal frequency and size), energy expenditure, body weight and body composition, feeding behavior, satiety, reward or hedonic consumption, and glucose homeostasis. Although the brain function in metabolic control has been explored for almost a century, the discovery of leptin and its cognate receptor in the mid-1990s and advances in molecular and genetic tools propelled the field toward an unprecedented development. In this chapter, we will highlight the main findings in recent years using these scientific tools with emphasis on the brain pathways and circuitry associated with the control of the metabolic function.

Keywords

Hypothalamus • Neuroendocrinology • Autonomic nervous system • Melanocortin • Lateral parabrachial nucleus • Paraventricular nucleus of the hypothalamus • Arcuate nucleus • Mesolimbic dopaminergic system

1 Introduction

The fundamental role of the central nervous system (CNS) in the regulation of feeding and energy homeostasis has been known for decades. Clinical observations in patients with Fröhlich's syndrome (adiposogenital dystrophy) displaying excessive subcutaneous fat due to adenopituitary tumors gave rise to an important debate on the relative contributions of the pituitary gland versus the overlying hypothalamus in the genesis of the metabolic aspects of the syndrome (Fröhlich 1901). While Fröhlich, Crowe, and Cushing supported the importance of the pituitary gland in the adiposity, Aschner, a few years later, demonstrated that removal of the pituitary gland alone did not affect adiposity in dogs, suggesting that damage of the hypothalamus was the main cause of the obese phenotype seen in Fröhlich's syndrome (Crowe et al. 1910; Aschner 1912; Elmquist et al. 1999). With the development of experimental tools to lesion restricted areas of the brain, Hetherington and Ranson reinforced Aschner's findings and proposed a crucial role for the hypothalamus in food intake and body weight regulation (Hetherington and Ranson 1940). They observed that bilateral lesions of the medial hypothalamus of rats (without disturbing the pituitary gland) produced a profound increase in body weight and adiposity. On the other hand, lesions of the lateral hypothalamic area induced hypophagia leading to death by starvation, in some cases (Anand and Brobeck 1951). Together, these observations gave rise to the classic "dual center" model proposed by Stellar in 1954, comprised of a "satiety center" (i.e., the ventromedial nucleus of the hypothalamus, the VMH) and a "feeding center" (i.e., the lateral hypothalamic area, the LHA) (Stellar 1954).

These ideas were later revised with the development of more refined and precise techniques. For example, small electrolytic or excitotoxic lesions of the VMH and adjacent areas, knife cuts of projecting fibers, and subdiaphragmatic vagotomy challenged the concept of the VMH as the satiety center (King 2006; Gold 1973; Cox and Powley 1981). Concurrently, others questioned the interpretation of data from lesions of the LHA due to the potential interruption of the medial forebrain bundle (which contains the ascending dopaminergic system), which might cause movement disorders or other behavioral changes (Stricker and Verbalis 1990; Bernardis and Bellinger 1996).

The discovery of the adipocyte-derived hormone leptin and its cognate receptor (LepRb) in the mid-1990s, together with the development of

new molecular and genetic tools, has permitted the identification of chemically defined neuronal populations associated with specific physiological components of energy homeostasis and the molecular dissection of relevant neural circuits (Zhang et al. 1994; Tartaglia et al. 1995; Chua et al. 1996; Lee et al. 1999; Myers and Leibel 2015).

As a starting point, the neural control of metabolic function recapitulates the basic organizational principles of the CNS in general. The sensory (*input*) arm perceives and conveys information on nutritional state and energy stores to specific brain nuclei (*integrative centers*) that integrate multiple physiological signals and orchestrate a coordinated response via the motor (*output*) arm. The sensory arm relies on hormones, peptides, and other signals from peripheral organs and tissues that function as "metabolic cues." In this chapter, we will summarize what we have learned in the past decade or so with the use of animal models and genetic tools. We will give special emphasis to the brain circuitry unraveled by studies performed in rodents, the preclinical animal model of choice in the field.

2 Sensing Metabolic Cues: Humoral and Neural Components

The sensory (input) arm of the neural control of the metabolic function may be subdivided in humoral and neural components, according to the route used by the metabolic cues to access the CNS. Most of these signals enter the CNS via the hypothalamus (mostly humoral signals) and brain stem (humoral and neural signals).

2.1 Humoral Components

Most metabolic cues derived from peripheral tissues are released into the circulation and directly act in specific hypothalamic and brain stem nuclei to control food intake, energy expenditure, and glucose homeostasis. Among them, hormones secreted by adipocytes (e.g., leptin), endocrine pancreas (e.g., insulin), and gut (e.g., ghrelin) have been widely investigated in the context of the neural control of the metabolic function.

Leptin, encoded by the *Lep/LEP* gene (previously called *ob* for obese), is primarily synthesized and secreted by white adipose tissue (Zhang et al. 1994). During negative energy balance, the fall in leptin levels represents a key signal for the neuroendocrine adaptations prompted by states of energy insufficiency (Flier 1998; Chan and Mantzoros 2005; Ahima et al. 2000; Casanueva and Dieguez 1999). These adaptive responses include decreased locomotor activity and thermogenesis, increased appetite and motivation for food, inhibition of the thyroid and reproductive axes, and activation of the adrenal axis (Ahima 2006). Leptin acts via LepRb (encoded by the *Lepr/LEPR* gene), which is highly expressed in several regions of the hypothalamus, including the arcuate nucleus (Arc), the VMH, the dorsomedial nucleus (DMH), and the LHA. In the brain stem, the ventral tegmental area (VTA), the periaqueductal gray matter, the lateral parabrachial nucleus (lPBN), and the nucleus of the solitary tract (NTS) also express LepRb (Tartaglia et al. 1995; Chua et al. 1996; Mercer et al. 1996; Fei et al. 1997; Elmquist et al. 1998a; Scott et al. 2009; Myers et al. 2009).

Insulin, produced by the pancreatic β cells, is crucial for the control of blood glucose; it stimulates glucose uptake by peripheral organs including liver, muscle, and adipose tissue (Weyer et al. 1999; Biddinger and Kahn 2006). Glucose uptake by neurons and glia is mediated by insulin-insensitive glucose transporters; hence, the acquisition and use of glucose by the brain are independent of insulin action (McEwen and Reagan 2004; Banks et al. 2012). Insulin receptors are widespread in the CNS, however, and growing evidence supports a role for brain insulin action in the control of energy balance (along with peripheral glucose homeostasis). For example, mice with neuronal deletion of insulin receptor display increased adiposity and higher susceptibility to obesogenic diet (Plum et al. 2006; Kleinridders et al. 2014; Bruning et al. 2000).

Ghrelin is primarily produced and released by endocrine cells of the stomach and small intestine

(Kojima et al. 1999). It was initially described as a potent growth hormone (GH) secretagogue, acting via the GH secretagogue receptor (GHSR). Soon after its discovery, several laboratories reported that peripheral or central injections of ghrelin potently stimulate food intake and decrease energy expenditure, leading to weight gain (Nakazato et al. 2001). Ghrelin is also an important modulator of glucose homeostasis. Loss-of-function mutations in the ghrelin gene (*Ghrl*) increase glucose-stimulated insulin secretion, as well as insulin sensitivity (Sun et al. 2006). Similarly, ghrelin infusion reduces insulin sensitivity and increases glucose levels. Some of ghrelin's actions may be mediated by direct effects in pancreatic islets (Dezaki et al. 2006), but many lines of evidence demonstrate major roles for GHSR in the brain (Nogueiras et al. 2008). GHSR is abundant in the Arc and VMH, as well as relevant brain stem sites, including the VTA, lPBN, and NTS (Nakazato et al. 2001; Zigman et al. 2006).

To exert their effects, circulating hormones must pass the blood–brain barrier (BBB) to access their receptors in the brain parenchyma. The BBB is composed of closely adjoined endothelial cells, glia, and (in some areas) tanycytes. It is present in the entire brain with the exception of small areas located adjacent to the cerebral ventricles, called circumventricular organs (CVOs). The CVOs contain fenestrated blood vessels that allow diffusion and interchange of bigger molecules (peptides and hormones) between the brain parenchyma and the bloodstream, presumably without the need for active transport across the BBB (Ganong 2000; Johnson and Gross 1993; Broadwell and Brightman 1976). Among the seven well-described CVOs, the median eminence and the area postrema are of particular interest here, given their proximity to metabolic sensing neurons in the Arc and the NTS, respectively. These are sites where metabolic signals may passively penetrate the brain and bind to receptors. Alternatively, hormones in the circulation may cross the BBB by two mechanisms: (a) via lipid-mediated free diffusion or (b) via carrier- or receptor-mediated active transport. Most of the metabolic hormones (e.g., leptin, insulin, and ghrelin) have BBB transporters that permit access to deep structures in the brain, not just CVO-adjacent regions (Banks et al. 1996, 2012; Balland et al. 2014; Banks 2008).

2.2 Neural Components: Visceral Inputs

The CNS control of energy homeostasis also relies on information conveyed by visceral inputs. Sensory information is generated in each segment of the alimentary tract, from food taste, temperature, and texture in the mouth to mechanical and chemical signals in the stomach and intestine. These signals are conveyed by several cranial nerves carrying different modalities of sensory inputs. The upper segments of the alimentary tract (mouth and tongue) convey gustatory inputs (taste signals) to the rostral subdivision of the NTS via the facial (VII) and glossopharyngeal (IX) cranial nerves; the mid- and lower segments (pharynx, larynx, esophagus, stomach, and intestine, as well as the liver and the portal vein) transmit mechanical and chemical inputs to the intermediary and caudal subdivisions of the NTS via the vagus (cranial nerve X). The different modalities of sensory inputs convey distinct information to the brain. For example, while gustatory inputs are associated with food selection and hedonic responses, mechano- and chemoreceptors signal nutritional content. In this regard, the vagus nerve is the primary neural component in the transmission of visceral inputs to the CNS (Chung and Andrea 2011; Pavlov and Tracey 2012).

The vagus nerve is comprised of afferent (sensory) and efferent (motor) fibers. The afferent vagal branch is organized as a typical sensory nerve, i.e., pseudounipolar neurons with cell bodies located in a ganglion outside the CNS, the nodose ganglion (aka inferior ganglion of the vagus nerve (Fig. 1)). Vagal dendrites, which contain specialized receptors, are distributed in a topographic manner along the mid- and lower segments of the alimentary tract. The mechanoreceptors are concentrated in the pharynx, esophagus, and stomach, and the chemoreceptors are

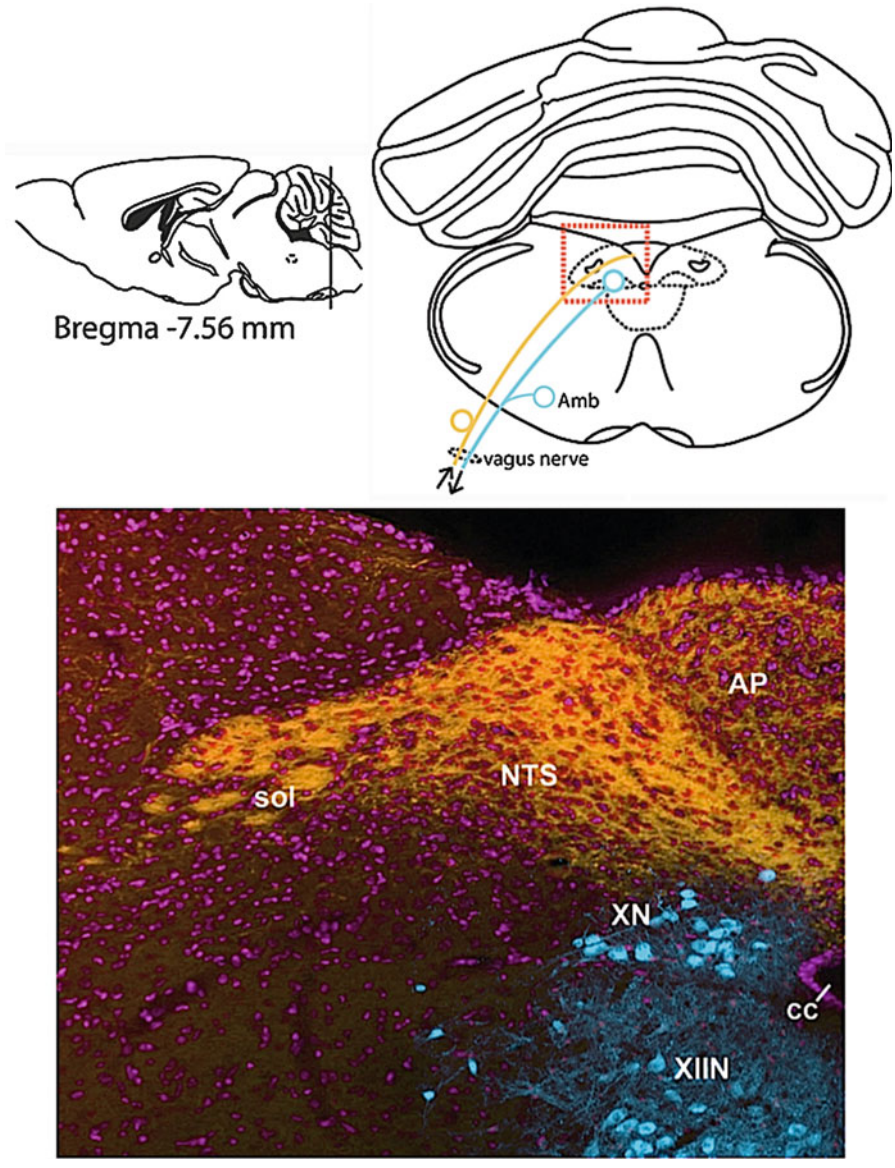

Fig. 1 Sensory and motor arms of the vagus nerve (*XN*). Sensory terminals innervate the area postrema (*AP*) and the nucleus of the solitary tract (*NTS*, pseudocolor *yellow*, using Nav1.8 reporter mice). Motor neurons in the motor nucleus of the vagus nerve (*DMV*) are represented in *blue* (choline acetyltransferase/ChAT-reporter mice). This image was kindly provided by Dr. Laurent Gautron from the University of Texas Southwestern Medical Center, Dallas, Texas, USA

more abundant in the stomach, liver, and intestine (Berthoud 2002). The mechanoreceptors are found throughout the myenteric plexus and external smooth muscle layers. In the stomach, they sense gastric distension and provide signals that promote satiation (Fox et al. 2001). The chemoreceptors are distributed in the mucosal and submucosal layers of the gastrointestinal (GI) tract and in the liver and portal vein; these may sense changes in glucose, amino acids, and fatty acids. The chemoreceptor cells are also responsive to peptides produced in the GI mucosa in response to food intake, including ghrelin, cholecystokinin (CCK), amylin, peptide YY, and

glucagon-like peptide-1 (GLP-1) among others in response to food intake (Berthoud 2002; Chaudhri et al. 2008). The viscerosensory inputs (via the vagus nerve) and humoral signals (via the area postrema) reach the CNS via direct actions upon NTS neurons. This neuronal relay functions as the primary brain stem entry site in metabolic regulation.

3 Brain Stem Pathways: Transducing Visceral and Humoral Inputs

Many humoral and neural signals of energy balance, including a variety of gut-derived signals, converge on the NTS (Fig. 2; Grill and Hayes 2012; Myers and Olson 2014). In addition to receiving the vagally encoded information from gut distension, a variety of humorally conveyed signals (including gut peptides, such as amylin and CCK) activate cells in the area postrema that project onto an overlapping set of neurons in the medial NTS; medial NTS responses contribute to short-term satiety. Additionally, many of the neurons in the medial NTS that receive vagal and area postrema-derived information also express LepRb and respond to leptin (Huo et al. 2008). Leptin augments the response of these cells to gut peptide- and vagally encoded signals, thus amplifying the effects of feeding on these satiety-promoting circuits (Huo et al. 2008; Morton et al. 2005).

The gut- and nutrient-responsive neurons of the medial NTS contain a variety of neurotransmitters; most are glutamatergic, but many also contain neuropeptide transmitters including proopiomelanocortin (POMC)-derived peptides, CCK, and GLP-1 (Huo et al. 2008; Garfield et al. 2012). Subpopulations of these cells express the transcription factor Phox2B, and Phox2b-Cre-mediated deletion of LepRb interferes with satiety signaling, as does virally mediated suppression of

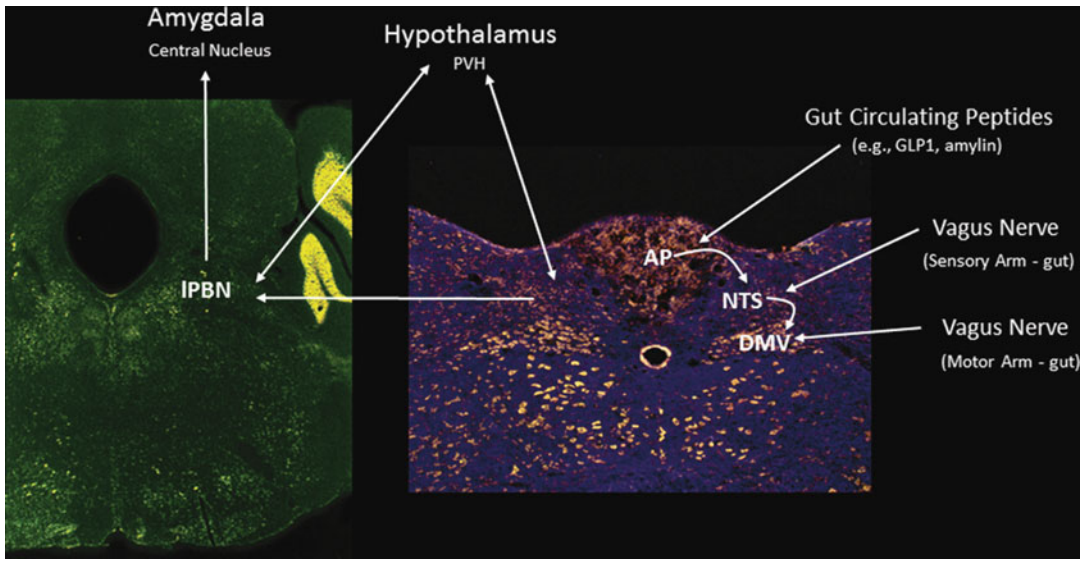

Fig. 2 Flow of information in the hindbrain. The area postrema (AP), a circumventricular organ that has direct access to the circulation, receives information about feeding status by sensing gut peptides (e.g., GLP1 and amylin). These cells project to the nucleus of the solitary tract (*NTS*), where the area postrema-derived information is integrated with information conveyed by vagal sensory afferents from the gut. This integrated information is not only passed to the dorsal motor nucleus of the vagus nerve (*DMV*) to stimulate vagal motor neurons efferent to the gut (controlling peristalsis, etc.) but is also passed forward to the lateral parabrachial nucleus (*lPBN*), an important center for anorexia, and to a variety of hypothalamic sites, including the paraventricular nucleus (*PVH*). Hypothalamic sites also project to the lPBN and NTS. The output of these nuclei promotes satiety. A subset of lPBN neurons projects to the central nucleus of the amygdala (CeA) to mediate a powerful anorectic signal

NTS LepRb expression (Aponte et al. 2011). Medial NTS neurons project to a variety of regions, including the adjacent dorsal motor nucleus of the vagus (DMV) – where they mediate gut reflexes that alter peristalsis, etc. (Grill and Hayes 2012). Medial NTS cells also project to the lPBN, where they synapse on neurons that contain calcitonin gene-related peptide (CGRP), among others (Wu et al. 2012). lPBN CGRP neurons project to the central nucleus of the amygdala to promote anorexia. Medial NTS neurons also make direct projections into more rostral areas, including hypothalamic sites (such as the paraventricular nucleus of the hypothalamus, the PVH), the amygdala, and the thalamus.

Importantly, in addition to roles played by brain stem nuclei in conveying gut- and nutrient-derived information rostrally, the NTS and lPBN receive information from hypothalamic structures and play an important role in mediating the control of food intake by these sites (Grill and Hayes 2012; Myers and Olson 2014). Both the NTS and lPBN receive direct inputs from the hypothalamus – especially from the PVH and the Arc nuclei; the lPBN plays prominent roles in the control of food intake by cells in both of these areas. Indeed, the lPBN also plays important roles in the anorexia associated with gut sickness-derived signals, as well as normal satiety signals.

4 Hypothalamic Systems that Control Energy Balance

4.1 Overall Organization

Many of the neural systems that control energy balance lie in the hypothalamus. Like the brain stem (and unlike more recently developed brain areas such as the neocortex and hippocampus), the hypothalamus is not organized in a laminar manner but rather consists of clusters of neuronal soma (nuclei). The cells within each nucleus connect to other cells in the same region and/or other nuclei to generate an integrated signal, which is then passed to output nuclei that ultimately relay the signal to motor neurons that control autonomic and endocrine systems or influence feeding behavior. While each of these nuclei contains heterogeneous (and even oppositely acting) types of neurons, the neurons of each nucleus often control related functions. While many hypothalamic nuclei contribute to the control of energy balance at some level, several of these nuclei play defined and especially important roles.

Within the medial region, the Arc, which lies immediately above the median eminence, enjoys rapid access to circulating factors (see Humoral Components discussed above) that mediate important signals of energy balance (Myers and Olson 2014). The Arc makes strong reciprocal connections with the dorsomedial nucleus of the hypothalamus (DMH), which integrates the Arc-derived signals with information (e.g., circadian cues, body temperature) from other hypothalamic regions (Fig. 3). The Arc and DMH each also make strong reciprocal connections with the PVH, which lies anterior to these other structures. As noted above, the PVH also receives direct input from the brain stem. The PVH represents a crucial output nucleus for the hypothalamus: PVH efferents to the brain stem and spinal cord control autonomic function, projections to the median eminence and posterior pituitary control endocrine function, and projections to the brain stem regulate feeding.

The VMH, especially the dorsomedial VMH (dmVMH, which mediates most of the energy balance function of the VMH), though nestled between the anterior portions of the Arc and DMH, makes relatively few connections with these two nuclei but rather projects to rostral and brain stem regions associated with autonomic function (e.g., the bed nucleus of the stria terminalis and the periaqueductal gray matter) (Canteras et al. 1994). Lateral to the Arc, VMH, and DMH lies a more loosely defined structure, the lateral hypothalamic area (LHA), through which course projections among several limbic regions rostral and caudal to the hypothalamus. The LHA contains many cell bodies, as well; many of these receive metabolic signals and project to the midbrain (including the dopaminergic ventral tegmental area or VTA) or to rostral limbic regions (such as the nucleus accumbens). The

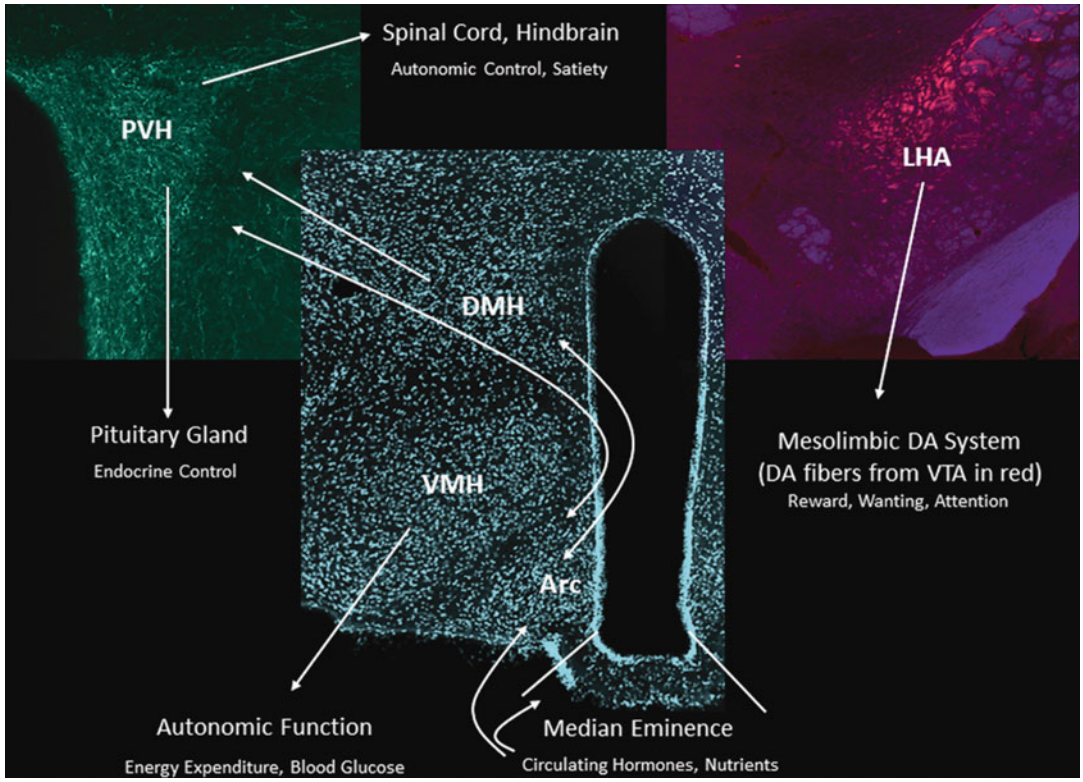

Fig. 3 Flow of information in the hypothalamus and roles of hypothalamic nuclei. The arcuate nucleus (*Arc*), which is located directly above the median eminence, has the most direct exposure to circulating hormones and nutrients and is enriched in receptors for these substances. Arc neurons, including the important POMC and AgRP neurons that comprise the inception site of the hypothalamic melanocortin system, project densely to the dorsomedial hypothalamic nucleus (DMH, where information is integrated with circadian, temperature, and other inputs) and to the paraventricular nucleus of the hypothalamus (PVH, the major output nucleus for the medial hypothalamus). The DMH makes reciprocal connections with the Arc and PVH. Projections from the PVH target the median eminence and pituitary gland for the regulation of endocrine function, the spinal cord to control sympathetic nervous system (*SNS*) function, and the hindbrain to modulate satiety. The ventromedial hypothalamic nucleus (*VMH*) senses glucose, along with some hormones that are sensed by the Arc, and projects to forebrain and hindbrain regions that control autonomic function, thereby controlling energy expenditure and blood glucose levels. The lateral hypothalamic area (*LHA*) contains many types of neurons that project into areas associated with attention, reward, and wanting, such as the mesolimbic dopamine system. The LHA represents a major conduit from hypothalamic homeostatic circuits into the brain's motivational circuitry

LHA represents a major conduit linking the hypothalamus to the mesolimbic dopamine system and other circuits that control motivation (Opland et al. 2010).

4.2 Arcuate Nucleus

The Arc in rodents (tuberal nucleus in humans) is located in the medioventral portion of the hypothalamus surrounding the third ventricle and intimately connected to the median eminence and hypophyseal portal vascular system by the infundibular stalk. It contains a heterogeneous group of projection neurons producing proopiomelanocortin (POMC), agouti-related peptide (AgRP), or Kisspeptin, hypophysiotropic neurons producing growth-hormone-releasing hormone (GHRH), somatostatin, or dopamine, plus glial-like tanycytes (in addition to astrocytes and microglia). A Golgi impregnation study showed that the majority of Arc neurons are bipolar with two major, relatively

aspiny dendrites (van den Pol and Cassidy 1982). Tanycyte cell bodies are located in the ventral-most ependymal lining of the third ventricle and elaborate their characteristic arching projections laterally and ventrally (Langlet 2014). The median eminence is one of the circumventricular organs with fenestrated capillaries and therefore provides neurons of the Arc with relatively unfettered access to circulating hormones, cytokines, nutrients, and metabolites. Modulation of tanycytes by peripheral signals, including leptin, appears to be capable of further opening the blood–brain barrier to allow access of circulating factors deeper into the Arc parenchyma (Balland et al. 2014; Mullier et al. 2010).

Neuropeptidergic neurons of the Arc, including those that contain POMC and somatostatin, are among the earliest differentiated neurons of the CNS, at E10.5 in the mouse. The homeodomain transcription factor Isl1 has recently been shown to be essential for the specification of POMC neuron identity and transcription of the *Pomc* gene by its interaction with two distinct neural-specific enhancers (Lam et al. 2015; Nasif et al. 2015). Similarly, the homeodomain transcription factor Bsx has been implicated in transcription of the *Agrp* gene (Sakkou et al. 2007). The early POMC-positive neurons in the developing Arc appear to be intermediate progenitors that ultimately give rise to mature POMC neurons, as well as subpopulations of mature AgRP and Kisspeptin neurons (Padilla et al. 2010; Sanz et al. 2015). Another defining feature of the developing Arc is the trophic action of the postnatal surge in leptin secretion to stimulate neural projections from the Arc to other hypothalamic nuclei (Bouret et al. 2004). There is increasing evidence that the epithelial lining of the third ventricle contains a stem cell population that together with tanycytes is capable of generating newly born and differentiated Arc neurons in the adult mouse brain (Kokoeva et al. 2007; McNay et al. 2012; Lee et al. 2012), although the physiological significance of these discoveries remains to be fully defined. Excitingly, recent reports have outlined the in vitro conditions essential to generate differentiated cells representative of the full range of Arc neurons from either human ES (embryonic stem) cells or IPSC (induced puripotential stem cells) cells (Merkle et al. 2015; Wang et al. 2015a). These groundbreaking findings will likely permit a more complete analysis of the factors underlying the development of the Arc and exploration of the genetic disturbances associated with Arc dysfunction in hereditary hypothalamic obesity syndromes.

Abundant genetic evidence from the clinic, together with animal studies, has identified the CNS melanocortin system, including POMC and AgRP neurons and their projections to distal neurons expressing the melanocortin MC3 and MC4 receptors, as a critical component of the homeostatic neural circuitry regulating energy balance (Cone 2005). Melanocortin peptides (α-, β-, and γ-MSH in humans, only α- and γ-MSH in rodents) are endogenous agonists of the two CNS receptors, while AgRP is a competitive antagonist/inverse agonist at both receptors (Ollmann et al. 1997). The remainder of this section will therefore focus on the dyad of Arc POMC and AgRP neurons.

A study by Cowley et al. (2001) first proposed a model to explain the homeostatic basis of body weight control, whereby leptin stimulation of POMC neurons is balanced by their inhibition from nearby AgRP neurons. In this useful (albeit simplistic) model, the two subpopulations of neurons largely project to similar sites in the CNS (Bagnol et al. 1999; Wang et al. 2015b) but have opposing actions, α-MSH ultimately leading to decreased food intake and increased energy expenditure and AgRP increasing food intake and decreasing energy expenditure. The model is conceptually very similar to the earlier, but now debatable, notion of opposing mediobasal hypothalamic anorexigenic and lateral hypothalamic orexigenic zones. More recent evidence has greatly elaborated on the dyadic model without nullifying its heuristic value.

AgRP neurons also produce neuropeptide Y (NPY) (Hahn et al. 1998) and the fast inhibitory neurotransmitter GABA (Cowley et al. 2001). Although AgRP and NPY both stimulate food intake following injection into the cerebrospinal fluid or specific hypothalamic nuclei, this pharmacological action does not fully reflect the complexity of endogenous AgRP neuron function.

Researchers were puzzled by the demonstration that mutant mice engineered to lack AgRP and/or NPY did not exhibit the predicted phenotype of decreased body weight, adiposity, and food intake (Qian et al. 2002). However, ablation of AgRP neurons in the adult, but not in neonatal mouse, causes starvation and death, and this phenotype is independent of melanocortin signaling (Luquet et al. 2005; Wu et al. 2008). These paradoxical findings have at least been partially explained by the primary role of GABA signaling from AgRP neurons in their acute actions to stimulate feeding, with the neuropeptides playing accessory or modulatory roles (Tong et al. 2008; Wu et al. 2009). Stimulation of AgRP neurons by either optogenetic or chemogenetic technology leads to the rapid onset of feeding behavior, even in sated mice (Aponte et al. 2011; Krashes et al. 2011). However, further experiments have demonstrated that endogenous AgRP release does indeed stimulate feeding but on a longer time scale than either GABA or NPY release (Krashes et al. 2013). There are apparently at least two parallel neural pathways mediating these effects, one a direct projection of AgRP neurons to the lPBN (Wu et al. 2012; Betley et al. 2013) and a second polysynaptic circuit involving an inhibitory AgRP projection to MC4R-expressing glutamatergic neurons within the PVH that in turn project by a descending pathway to the lPBN (Garfield et al. 2015). It is not yet known if the latter target neurons are identical to each other for both pathways originating from the Arc.

Like AgRP neurons, subpopulations of Arc POMC neurons are characterized by diverse afferent signaling pathways, including activation by the humoral factors leptin and insulin via LepRb and InsR (Qiu et al. 2010, 2014), respectively, either direct activation or inhibition by glucose (Ibrahim et al. 2003; Parton et al. 2007), transsynaptic excitation by glutamatergic inputs (Kiss et al. 2005; Sternson and Shepherd 2005), and transsynaptic inhibition by GABAergic inputs (including those from local AgRP/NPY/GABA neurons), opioid peptides via μ-opioid receptors (Pennock and Hentges 2011), and serotonin via 5-HT2C receptors (Berglund et al. 2013). POMC neurons also synthesize cocaine- and amphetamine-regulated transcript (CART) and dynorphin peptides and are capable of the synaptic release of both GABA and glutamate (Hentges et al. 2009; Jarvie and Hentges 2012), although the physiological importance of these co-modulators and co-transmitters is still unknown. However, there is abundant and unequivocal evidence from pharmacological, genetic, and electrophysiological experiments that melanocortin peptides derived from POMC neurons play a critical physiological role in the reduction of food intake by promoting early satiation and in the reduction of energy expenditure via effects on the autonomic nervous system (Yaswen et al. 1999; Huszar et al. 1997; Butler et al. 2000; Chen et al. 2000; Xu et al. 2011). The actions of β-endorphin, an opioid peptide that is generated stoichiometrically with melanocortins during posttranslational processing of the prohormone, are less certain (Wardlaw 2011). β-Endorphin injected into the nucleus accumbens acutely stimulates feeding, particularly of highly palatable food (Majeed et al. 1986; Will et al. 2003); however, mice with a specific genetic loss of β-endorphin exhibit a mild obesity phenotype with increased food intake (Appleyard et al. 2003). The explanation for these contradictory findings has not been adequately explained.

Unlike the rapid stimulatory effects on food intake produced by the remote activation of AgRP neurons, activation of Arc POMC neurons has only produced delayed inhibitory effects on food intake after as much as 24 h (Aponte et al. 2011; Atasoy et al. 2012; Zhan et al. 2013). Similarly, only long-term inhibition of POMC neurons was capable of increasing food intake (Atasoy et al. 2012). Because the feeding inhibitory effects from optogenetic activation of POMC neurons were blocked by a melanocortin antagonist and POMC neuron activation could overcome coincident inhibition from AgRP neurons, the most parsimonious explanation for the delayed response is that melanocortin peptide release, and not the other putative peptide and amino acid transmitters produced in POMC neurons, is of principal importance to POMC neuron function in the control of energy homeostasis. However, it is worth noting that the loss of *Pomc* gene

expression selectively from the Arc has no detectable effect on body weight in mice until they are weaned at age 3 weeks (Bumaschny et al. 2012). The absence of *Pomc* expression also does not prevent the maturation of POMC neurons and the development of their widespread axonal projections throughout the brain.

The most recent advances in our understanding of the intrinsic activity of POMC and AgRP neurons and their role in energy balance come from a pair of elegant studies using in vivo Ca^{2+} imaging of the Arc with either fiber photometry or miniaturized confocal optics in freely behaving mice (Chen et al. 2015; Betley et al. 2015). These experiments revealed unexpected aspects of neuronal activity that have not been possible to assess using ex vivo slice electrophysiology. Fasted mice were shown to have tonically active AgRP neurons and tonically inhibited POMC neurons. Food presentation alone or even olfactory cues from a hidden food pellet were sufficient to immediately reverse the activity state of the two populations of Arc neurons. Furthermore, the magnitude of these responses was increased directly with the hedonic value of the presented food, and, conversely, food removal slowly restored the original activation states. Extrinsic excitation of the AgRP neurons conditioned mice to avoid a previously associated neutral flavor or preferred chamber in a place preference test. These results suggest that AgRP neuron activation has a negative valence, and, as a corollary, the state of food deprivation associated with tonically active AgRP neuron firing is intrinsically aversive. Therefore, the natural drive to eat, with consequent achievement of reward, may be motivated not only by the positive hedonic reinforcement from food but also by the reduction of negative reinforcement encoded by AgRP neuron activity. Although not explicitly tested, it is logical to propose that POMC neuron activation may have an opposing positive valence that is unrelated to its induction of satiation. Finally, these demonstrations of rapid alterations in Arc neuronal firing in anticipation of food consumption, rather than as a response to it, provide new insights concerning the role of the melanocortin system in both homeostatic and non-homeostatic control of energy balance.

4.3 Dorsomedial Nucleus

Even among the complex nuclei of the hypothalamus, the size and functional diversity of the DMH is substantial (Fontes et al. 2011; Dimicco et al. 2007). While there are many recognized subdivisions of the DMH, it is probably most useful to distinguish among the dorsal component (DMHd, which borders the dorsal hypothalamic area or DHA), the compact central zone, and the ventral region (DMHv). The DMH plays a role in the control of many autonomic functions, including thermogenesis, heart rate, and blood pressure. Like the Arc, the DMH contains a substantial number of LepRb-expressing cells (Scott et al. 2009; Patterson et al. 2011). Dorsal DMH/DHA LepRb neurons interact with the thermal control systems of the medial preoptic area and PVH and play an important role in the control of body temperature by leptin (Rezai-Zadeh et al. 2014). Consistently, deletion of LepRb in the prolactin-releasing hormone-expressing neurons of this region decreases body temperature and energy expenditure, promoting obesity in high-fat-fed animals (Dodd et al. 2014). A variety of data also suggest that the LepRb neurons in this region modulate blood pressure and contribute to the increase in blood pressure associated with the hyperleptinemia of obesity (Simonds et al. 2014).

Some brain lesion experiments also suggest a role for the DMH in the control of food intake. It is possible that the DMH contains oppositely acting sets of neurons (similar to the POMC and AgRP neurons of the Arc but more evenly balanced), which could limit the ability to detect roles in the control of food intake following traditional lesioning. It is also possible that the variable effects on feeding that result from DMH lesions may reflect differences in the subregions of the DMH targeted in various studies; certainly, DMHd/DHA LepRb neurons control autonomic output but do not modulate feeding (Rezai-Zadeh et al. 2014). Correlative evidence suggests the potential for DMH leptin action (presumably the ventral DMH) in the control of feeding, however. The deletion of LepRb from distributed populations of hypothalamic cells that express the vesicular GABA transporter

(vGat, *Slc32a1* gene) or neuronal nitric oxide synthase (nNOS, *Nos1* gene) each produces dramatic hyperphagia and obesity (Leshan et al. 2012; Vong et al. 2011). The distributions of these cells overlap mainly in the DMH, suggesting a potential role for DMH LepRb neurons in the suppression of feeding.

4.4 Paraventricular Nucleus: Hypothalamic Output

The PVH is a critical hypothalamic center that receives and integrates energy balance signals from a variety of brain regions and coordinates physiologic responses to maintain energy homeostasis predominantly through the autonomic nervous system. The PVH is a complex structure composed of a heterogeneous group of mostly glutamatergic neurons that have been classically described as parvocellular or magnocellular based on cell size and axonal projection patterns. The magnocellular neurons in the PVH, including those that express oxytocin (OXT) or vasopressin (AVP), project primarily to the posterior pituitary and release their contents directly into the general circulation to regulate peripheral tissue function. Importantly, however, dendritic release of these neuropeptides has been implicated in the overall control and coordination of PVH function.

The PVH parvocellular cells are more diverse and send projections within the central nervous system to three main areas: (1) the median eminence where secreted factors (e.g., corticotropin-releasing hormone or CRH) enter the portal hypophyseal circulation and regulate pituitary function; (2) the brain stem, including the dorsal vagal complex (composed of the NTS and DMV) and the lPBN – both of which have been implicated in feeding (Wu et al. 2009; Wan et al. 2008; Wu and Palmiter 2011; Zheng et al. 2005; Berthoud et al. 2006); and (3) the preganglionic, sympathetic output centers such as the intermediolateral cell column of the spinal cord (Sawchenko and Swanson 1982; Swanson et al. 1980; Biag et al. 2012). Parvocellular PVH neurons that respond to satiety signals, such as leptin, have been proposed to regulate feeding by modulating hindbrain responses to ascending feeding signals from the gut and periphery (Morton et al. 2005; Atasoy et al. 2012; Blevins et al. 2004, 2009). However, it is important to point out that hypothalamic factors secreted into the portal hypophyseal circulation at the median eminence undoubtedly contribute to both energy and metabolic homeostasis via regulation of pituitary function.

The overall importance of the PVH in the regulation of energy balance is underscored by the massive obesity and metabolic abnormalities associated with alterations in PVH development or function. Rodents and humans harboring deleterious mutations in the hypothalamic transcription factor *single minded-1* (Sim1) develop a hypocellular PVH and hyperphagic obesity. Moreover, lesions of the PVH also result in hyperphagic obesity and glucose dysregulation. Neither the neural architecture nor the molecular mechanisms used by the PVH to maintain energy and metabolic homeostasis are well understood. This is in large part due to the cellular heterogeneity of the PVH, the density of its projection targets, and the array of PVH afferent inputs from different brain regions (Sawchenko and Swanson 1983; Ferguson et al. 2008).

The PVH serves as an important regulatory output center for peptides and conditions known to modulate food intake, including leptin, melanocortins (from the Arc), GLP-1 (presumably from the NTS), GLP-1 agonists, and dehydration (Tung et al. 2008; Acuna-Goycolea and van den Pol 2004; Baraboi et al. 2011; Dalvi et al. 2012; Salter-Venzon et al. 2008). The melanocortin system is perhaps the best studied of these pathways, as it is essential for energy balance in rodents and humans and is directly linked to PVH function (Cone 2005; Garfield et al. 2015; Farooqi and O'Rahilly 2006; Farooqi et al. 2003). POMC and AgRP neurons in the Arc produce melanocortin agonists and antagonists, respectively, and project to PVH neurons that express melanocortin receptors (Ellacott and Cone 2004; Kishi et al. 2003; Mountjoy 2010). Endogenous and pharmacologic melanocortin agonists stimulate melanocortin receptor-bearing neurons to activate effector pathways that inhibit food intake and stimulate energy

expenditure. Melanocortin action in PVH Sim1 neurons suppresses food intake (Balthasar et al. 2005; Shah et al. 2014), and ablation of most Sim1 neurons in adult mice results in profound hyperphagic obesity with decreased energy expenditure and altered locomotor activity (Xi et al. 2012). In addition, selective deletion of MC4R from Sim1 cells leads to hyperphagic obesity (Shah et al. 2014).

Subsets of PVH neurons contain a variety of neuropeptides implicated in neuroendocrine and energy balance control, including OXT, CRH, AVP, thyrotropin-releasing hormone, and somatostatin. The anorectic effects of pharmacologic doses of OXT and CRH agonists generated a great deal of interest in PVH OXT and CRH neurons as potential regulators of energy balance. At odds with this formulation are the findings that rodents lacking OXT or OXT neurons (or CRH/CRH receptors) demonstrate minimal energy balance phenotypes; neither does the activation of PVH OXT or CRH neurons alter feeding (Sutton et al. 2014). Whether the contradiction between pharmacologic studies and genetic approaches reflects developmental compensation to the systemic inactivation of these neuropeptides is not clear, but the profound effects of Sim1 neuron (pan-PVH) manipulation suggest that yet-to-be-defined PVH neurons distinct from OXT and CRH cells represent crucial mediators of energy balance.

With the recent development of an array of genetic tools, cell-specific genetic changes in PVH cells have confirmed the critical role of the PVH in feeding regulation and have extended our understanding of the molecular components and neural circuitry of PVH function/action. MC4R action on Sim1 cells in the PVH is sufficient to normalize feeding in animals that lack MC4Rs elsewhere, and this is not attributable to direct MC4R action on OXT, CRH, or AVP neurons. Moreover, MC4R expression in Sim1 PVH neurons is required for body weight maintenance, indicating that PVH MC4R action is both necessary and sufficient for normal energy homeostasis (Balthasar et al. 2005; Shah et al. 2014). Remote activation of Sim1 PVH neurons using chemogenetic approaches suppresses feeding and increases energy utilization (Garfield et al. 2015; Sutton et al. 2014). The effects of pan-PVH activation on parameters of energy balance are not assignable to PVH OXT, CRH, or AVP neurons, since chemogenetic manipulation of these populations had minor (if any) effects on energy balance. In contrast, cell-specific activation of neuronal nitric oxidase synthase (NOS1)-expressing PVH neurons (a subset of Sim1 PVH cells) alters feeding to a similar extent as pan-PVH activation, suggesting that PVH NOS1 neurons play an important role in feeding.

The PVH sends projections to a variety of brain regions within the central nervous system. For the purposes of this discussion, we will highlight the functional roles of PVH projections to brain areas known to be important for food intake/energy expenditure, including the lPBN (feeding), NTS, and spinal cord (autonomic control). The importance of these specific PVH projections has been inferred based on published data demonstrating the importance of these target regions in energy balance. The combination of stereotaxic delivery of cell-specific viral tools into transgenic animals with technologies such as light-dependent neural activation (optogenetics) has made it possible to interrogate the physiologic function of specific PVH neuronal projections. Indeed, recent studies using these technologies have revealed a PVH → Arc orexigenic circuit and established the importance of PVH → lPBN projections for melanocortin action in the CNS (Garfield et al. 2015; Krashes et al. 2014). Similar approaches targeting other PVH projections will undoubtedly uncover additional important biological mechanisms underpinning energy balance regulation.

4.5 Ventromedial Nucleus

For several decades following the seminal studies of Hetherington and Ranson, in which the bilateral medial hypothalamic lesions (that included the VMH) produced hyperphagic obesity, the VMH was the main focus of attention regarding the neural control of energy homeostasis (Hetherington and Ranson 1940). Following

intense debate, these studies were dismissed by findings suggesting that the electrolytic lesions likely disrupted the neural connections of the medial hypothalamus, including projections from the Arc to and from the PVH (Elmquist et al. 1999; King 2006). While still not completely resolved, the role for the VMH in energy balance has been clarified by recent studies using more specific molecular and cellular methods.

The VMH contains glucose-sensing neurons that are highly responsive to changes in glucose levels, as well as those that express receptors for metabolic hormones (e.g., leptin and insulin) or for neuropeptides associated with energy balance (Scott et al. 2009; Elmquist et al. 1997; Routh 2003; Song et al. 2001; Kang et al. 2004). However, the VMH, like most hypothalamic nuclei, is not a homogeneous structure, and it is comprised of neurons with distinct neurochemical identities and characteristic projection patterns. For example, the ventrolateral subdivision (VMHvl) expresses sex steroid receptors and projects to sites related to behavioral control, whereas neurons in the dorsomedial subdivision (VMHdm) respond to metabolic cues (e.g., glucose, leptin, and insulin) and innervate areas associated with autonomic and circadian regulation (Canteras et al. 1994; Kim et al. 2011a; Elmquist et al. 1998b; Klockener et al. 2011). Among these, VMHdm projects densely to the lateral aspect of the bed nucleus of the stria terminalis and to the subparaventricular zone of the hypothalamus (Canteras et al. 1994; Elmquist et al. 1998b; Dong and Swanson 2004). The lateral bed nucleus of stria terminalis is part of the central autonomic circuitry preferentially innervating the central amygdala, periaqueductal gray matter, lPBN, and NTS (Dong and Swanson 2004). On the other hand, the subparaventricular zone receives dense innervation from the suprachiasmatic nucleus, the main circadian clock of the mammalian brain (Moore 1983; Watts et al. 1987). The neuroanatomical organization of the VMHdm suggests roles in energy balance by the control of autonomic function (e.g., thermogenesis, hepatic glucose production, glucose utilization, and secretion of insulin and glucagon) and the circadian oscillations of circulating hormones (e.g., corticosterone) in response to changes in energy stores (Kim et al. 2011a; Bernardis and Frohman 1971; Luo et al. 1999; Niijima et al. 1984; Krieger 1980; Choi et al. 1996).

To better understand the regulation and function of VMH circuits, several groups examined the temporal and anatomic distribution of gene expression in the VMH. Of the identified genes, steroidogenic factor 1 (SF1, *Nr5a1* gene) received a great deal of attention due to its VMH-specific expression within the CNS (Ikeda et al. 1995; Segal et al. 2005). The restricted expression of SF1 has allowed the development of a series of genetically modified mouse models to interrogate VMHdm function. Mice with global loss-of-function mutations in the SF1 gene show disrupted VMH development (Ikeda et al. 1995; Sadovsky et al. 1995; Luo et al. 1994; Shinoda et al. 1995), and neuron-specific deletion of SF1 results in morbid obesity – primarily due to decreased energy expenditure (Kim et al. 2011a, b; Majdic et al. 2002). Two independent groups also demonstrated that leptin signaling in VMH SF1 neurons is required for energy expenditure, glucose homeostasis, and adaptive thermogenesis and hence for the control of body weight (Dhillon et al. 2006; Bingham et al. 2008). On the other hand, selective deletion of insulin receptor from SF1 neurons induced resistance to obesogenic diet and altered glucose metabolism (Klockener et al. 2011).

The VMH also plays a prominent role in the control of glucose homeostasis (Routh 2003). VMH neuron responses to glucose are heterogenous: Glucose-excited (GE) VMH neurons increase and glucose-inhibited (GI) VMH neurons decrease their firing rate when glucose rises (Song et al. 2001). Intra-VMH 2-deoxyglucose (a non-metabolizable glucose analog that mimics low glucose) injection increases plasma glucose, glucagon, noradrenaline, and adrenaline, suggesting a role in the counter-regulatory response to hypoglycemia, presumably by activating GI neurons. Recent studies have also suggested that VMH innervation by brain stem sites also plays a role in this response (Garfield Alastair et al. 2014; Flak et al. 2014). Conversely, leptin

or melanocortin action in the VMH increases glucose uptake into tissues and/or decreases blood glucose (presumably via GE neurons). These findings support the notion that the VMH is a key component in the control of glucose homeostasis via sensing changes in glucose levels and modulation of autonomic responses (Routh 2003).

Targeted deletion of leptin- or insulin-regulated intracellular signaling pathways has unraveled some of the molecular mechanisms involved in the humoral control of the VMH function. For example, reduced activity of phosphatidylinositol 3-kinase (PI3K) in SF1 neurons decreased the adaptive autonomic response to high caloric intake without changes in glucose homeostasis (Xu et al. 2010). In addition, deletion of FOXO1 (a transcription factor downstream of PI3K signaling) resulted in improved insulin sensitivity and in a lean phenotype due to increase in energy expenditure (Kim et al. 2011b).

Non-SF1 neurons (such as those that contain brain-derived neurotrophic factor or BDNF) may also play an important role in metabolic regulation: Viral blockade of VMH BDNF induces hyperphagic obesity in mice (Unger et al. 2007). Also, deletion of estrogen receptor α (ERα) in the entire VMH results in more profound adiposity than that observed in selective ERα deletion from SF1 neurons (Xu et al. 2011; Musatov et al. 2007). Hence, VMH SF1 neurons appear to primarily control energy expenditure and glucose homeostasis, whereas non-SF1 neurons may have a more prominent role in the regulation of food intake. The specific neural pathways that lie downstream of distinct VMH neurons to control each aspect of energy homeostasis remain poorly understood, however.

4.6 Lateral Hypothalamic Area and Mesolimbic Dopaminergic System

Unlike the control of autonomic and endocrine function, foraging for and eating food require the initiation and coordination of complex behaviors. While the neural circuits that generate motor patterns represent the ultimate outputs for these behaviors, these circuits serve the brain's motivational systems (Berthoud 2007). The central control of motivation is mediated by the mesolimbic dopamine (DA) system, at the core of which lie DA neurons of the midbrain ventral tegmental area (VTA) (Berridge 2004). The VTA DA neurons project to many places, including the nucleus accumbens, where DA release modulates motivation.

Several decades-old observations also suggested a role for the LHA in motivation. Not only does lesioning the LHA promote anhedonia and abrogate the motivation to feed in experimental animals, but also animals will self-administer activating current to the LHA, suggesting that LHA activation is rewarding/motivating (Fulton et al. 2000). Since the medial forebrain bundle, which carries axons from (among others) the VTA to the nucleus accumbens, courses through the LHA, it was not initially clear whether the perturbation of the medial forebrain bundle or rather LHA neurons mediate these effects, however.

With the discovery and functional characterization of several discrete sets of LHA neurons, it became clear that LHA neurons themselves play an important role in the control of motivation, including the mesolimbic DA system. Indeed, the LHA integrates metabolic (e.g., leptin and melanocortins) and other homeostatic signals from the hypothalamus to modulate mesolimbic DA-dependent activity, attention, and motivation (Myers et al. 2009; Berthoud 2007). Like the Arc, VMH, and DMH, the LHA contains many groups of neurons, some of which function antagonistically. Important LHA neurons include orexin (aka hypocretin)-containing cells, which project to a variety of midbrain and hindbrain sites. Orexin neurons are activated by signals of energy deficit (fasting, ghrelin, etc.) to promote arousal and food seeking; conversely, leptin inhibits orexin neurons (Myers et al. 2009; Berthoud 2007). Melanin-concentrating hormone (MCH)-expressing neurons in the LHA project widely through the forebrain, including to the nucleus accumbens, and stimulate feeding (Georgescu et al. 2005). Overexpression of MCH promotes increased feeding and weight gain, while the ablation of MCH or MCH neurons decreases feeding and promotes leanness.

While both orexin and MCH neurons are glutamatergic, the LHA also contains a substantial population of GABAergic neurons, many of which contain neuropeptides, including neurotensin and galanin. While orexin neurons contain GHSR (the receptor for orexigenic ghrelin), neither orexin nor MCH cells contain LepRb (Leinninger et al. 2009). Rather, a substantial subset of LHA GABA neurons coexpresses LepRb. Like the larger population of LHA GABA neurons, many LHA LepRb neurons also contain neurotensin and/or galanin (Laque et al. 2013).

LHA LepRb neurons contribute to the control of feeding, energy expenditure, and energy balance by leptin, since intra-LHA leptin suppresses feeding in leptin-deficient animals, and deletion of LepRb in LHA neurotensin neurons decreases activity and energy expenditure, while increasing adiposity (Leinninger et al. 2011). LHA LepRb neurons project locally onto orexin (but not MCH) neurons, in addition to innervating the VTA and other midbrain sites (Louis et al. 2010). While the action of Arc-derived melanocortins apparently drives the control of MCH neurons by leptin and energy balance, leptin action via LHA LepRb neurons inhibits the activity of orexin neurons, at least in part via galanin (Opland et al. 2010; Goforth et al. 2014). LHA leptin action also promotes the expression of orexin; this somewhat counterintuitive bidirectional regulation of orexin neurons presumably reflects the need for leptin to reduce acute foraging activity, while supporting the normal function of orexin to permit alertness and attention (Louis et al. 2010).

Leptin action via LHA neurotensin neurons also modulates the mesolimbic DA function, decreasing nucleus accumbens DA transport activity (hence increasing synaptic DA transmission) (Leinninger et al. 2011). Since the chemogenetic activation of LHA neurotensin neurons increases nucleus accumbens DA concentration via the release of neurotensin in the VTA, intra-VTA neurotensin release by LHA LepRb neurons presumably represents a mechanism by which LHA LepRb neurons control mesolimbic DA function (Patterson et al. 2015). While LHA LepRb neurons represent a major mechanism by which leptin and energy status control the mesolimbic DA system, some VTA cells also contain LepRb (Leshan et al. 2010; Fulton et al. 2006; Hommel et al. 2006). VTA LepRb neurons mainly project locally and to the central nucleus of the amygdala (rather than the nucleus accumbens), and the ablation of LepRb from DA neurons fails to alter energy balance or tested parameters of mesolimbic DA function. Thus, LHA LepRb neurons represent the primary link between leptin and the control of mesolimbic DA function and motivation.

5 Conclusions

The physiological regulation of varied components of the metabolic function relies on a coordinated action of humoral and neural signals, integrated brain circuits and orchestrated motor, and behavioral and reflex responses. With the use of molecular and genetic tools and new technology, we have a much clear picture of the role of specific neuronal populations and brain pathways associated with the control of many aspects of energy homeostasis. The "dual center" hypothesis has its heuristic value, but recent evidence using more precise tools has demonstrated the complexity of the system with the action of a series of hypothalamic and brain stem nuclei. We have also gained knowledge on the relevance of selective neurotransmitters/peptides and neural pathways in several aspects of the metabolic regulation. The next challenge will be to determine how each of these components is interconnected and integrated to generate a highly coordinated physiological system.

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6 Cross-References

- Adipokines and Metabolism
- Adipose Structure (White, Brown, Beige)
- Bariatric Surgery
- Circadian Rhythms and Metabolism
- Genetics of Obesity
- Gut Hormones and Obesity
- Insulin Resistance in Obesity
- Overview of Metabolic Syndrome
- Pancreatic Islet Adaptation and Failure in Obesity and Diabetes
- Pharmacotherapy of Obesity and Metabolic Syndrome

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