Hypothalamic and brainstem neuronal circuits controlling homeostatic energy balance

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Abstract

Alterations in adequate energy balance maintenance result in serious metabolic disturbances such as obesity. In mammals, this complex process is orchestrated by multiple and distributed neuronal circuits. Hypothalamic and brainstem neuronal circuits are critically involved in the sensing of circulating and local factors conveying information about the energy status of the organism. The integration of these signals culminates in the generation of specific and coordinated physiological responses aimed at regulating energy balance through the modulation of appetite and energy expenditure. In this article, we review current knowledge on the homeostatic regulation of energy balance, emphasizing recent advances in mouse genetics, electrophysiology, and optogenetic techniques that have greatly contributed to improving our understanding of this central process.

Key Words
- CNS
- ghrelin
- leptin
- neuroendocrinology
- obesity

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Introduction

The regulation of appetite and body weight is an intricate process controlled by redundant and distributed neural systems that integrate a myriad of cognitive, hedonic, emotional, and homeostatic cues to precisely regulate systemic energy balance through behavioral, autonomic, and endocrine outputs. These sophisticated biological programs are influenced by multiple factors, including environmental, genetic, and epigenetic mechanisms. The immense complexity of these systems illustrates the biological importance of adequate nutrient and energy balance, a process that has been evolutionarily conserved and refined to guarantee appropriate adiposity levels. Despite the precision of these systems in matching energy demand with energy expenditure, contemporary, and lifestyle factors are the main causes of the prevailing obesity epidemics. The present review attempts to summarize current understanding of the anatomy, neurochemistry, functions, and interactions of relevant neural circuits involved in the homeostatic regulation of energy balance.

The homeostatic system: hypothalamus and brainstem

The hypothalamus: neuronal anatomy, nuclei, and neuropeptides

Seminal lesioning studies conducted in rodents during the 1940s and 1950s highlighted the importance of the hypothalamus in the regulation of body weight. Since then, extensive experimental evidence and extraordinary

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progress in understanding the neurobiology of obesity have firmly established the mediobasal hypothalamus as a fundamental nexus in the neuronal hierarchy controlling whole-body energy balance. The hypothalamus is constituted by distinct hypothalamic nuclei including the arcuate nucleus (ARC), the paraventricular nucleus (PVN), the lateral hypothalamic area (LHA), the dorsomedial nucleus (DMN), and the ventromedial nucleus (VMN).

Arcuate nucleus The ARC is a very important area of the CNS involved in the control of energy homeostasis. It is located below the VMN, on both sides of the third ventricle, and immediately adjacent to the median eminence (ME). This area has a semi-permeable blood–brain barrier (BBB; Broadwell & Brightman 1976), and thus it is strategically positioned to sense hormonal and nutrient fluctuations in the bloodstream. In the ARC, there are at least two major populations of neurons controlling appetite and energy expenditure: i) a subset of neurons that coexpress orexigenic neuropeptide Y (NPY) and agouti-related peptide (AGRP) and ii) a population of neurons that coexpress the anorexigenic neuropeptides cocaine- and amphetamine-regulated transcript (CART (CARTPT)) and α-melanocyte-stimulating hormone (α-MSH, a product of proopiomelanocortin (POMC) processing). These two populations of neurons (hereafter referred to as AgRP and POMC respectively), together with downstream target neurons expressing the melanocortin receptor 4 (MC4R) and MC3R, constitute the central melanocortin system. This neuronal circuit is crucial for sensing and integrating a number of peripheral signals allowing for a precise control of food intake and energy expenditure (see section 'ARC neuronal circuits: POMC, AgRP, and RIPCre neurons').

NPY is widely expressed throughout the CNS, but it is most densely localized in the ARC in the hypothalamus (Gehlert *et al*. 1987). The expression and release of ARC NPY respond to changes in energy status, being reduced under feeding conditions and increased under fasting conditions (Beck *et al*. 1990, Kalra *et al*. 1991). Increasing NPY tone pharmacologically results in hyperphagia and reduced thermogenesis of brown adipose tissue (BAT), associated with diminished activity of the thyroid axis (Clark *et al*. 1984, Stanley *et al*. 1986, Egawa *et al*. 1991). Although NPY acts at five different receptors (Y1, Y2, Y3, Y4, and y6), genetic and pharmacological studies suggest that postsynaptic Y1 and Y5 receptors mediate the effects of NPY on positive energy balance (Nguyen *et al*. 2012, Sohn *et al*. 2013).

AGRP is also an orexigenic neuropeptide that is exclusively expressed in the ARC, where it colocalizes with NPY and the neurotransmitter γ-aminobutyric acid (GABA; Broberger *et al*. 1998, Cowley *et al*. 2001). The central administration of AGRP or its genetic overexpression stimulates food intake, reduces energy expenditure, and causes obesity (Graham *et al*. 1997, Ollmann *et al*. 1997, Small *et al*. 2003). Interestingly, lasting orexigenic effects (over days) after AGRP delivery have been reported (Hagan *et al*. 2000).

AgRP neurons express receptors for peripheral hormonal signals such as insulin (Marks *et al*. 1990), leptin (Elmquist *et al*. 1998), and ghrelin (Willesen *et al*. 1999). These neurons send projections mainly into the PVN, DMN, and LHA. Despite the well-documented effects of NPY and AGRP as positive modulators of energy balance, genetic studies have yielded conflicting results. For example, *Agrp*- and *Npy*-knockout (KO) mice failed to exhibit alterations in body weight or feeding behavior (Palmiter *et al*. 1998, Qian *et al*. 2002, Corander *et al*. 2011). However, the ablation of AgRP neurons in adults leads to uncontrolled anorexia but is well tolerated in neonates, indicating the existence of developmental compensations (Bewick *et al*. 2005, Gropp *et al*. 2005, Luquet *et al*. 2005).

CART is widely expressed in the brain, but it is particularly abundant in the hypothalamus, and it colocalizes (>95%) with POMC in the ARC (Elias *et al*. 1998). Its expression is enhanced under feeding conditions and reduced under fasting conditions (Kristensen *et al*. 1998), and it has been shown that i.c.v. infusion of CART inhibits food intake, while antibodies against CART reverse this effect (Kristensen *et al*. 1998). Furthermore, CART also stimulates the thermogenesis of BAT (Kotz *et al*. 2000). However, *Cartpt*-deficient mice exhibit no alterations in food intake or body weight when fed with a standard diet, but develop obesity after being fed with a high-fat diet (HFD; Asnicar *et al*. 2001). Interestingly, and contrary to the prevailing anorexigenic view, other studies have shown that under certain experimental conditions CART may stimulate food intake (Abbott *et al*. 2003, Kong *et al*. 2003). Collectively, results regarding the effects of CART on feeding behavior are inconclusive and indicate anatomically divergent roles for this neuropeptide.

POMC is a prohormone precursor that is cleaved into several bioactive peptides in the hypothalamus, including α-MSH, which exerts potent anorexigenic effects by binding to MC3R and MC4R (Mercer *et al*. 2013). The levels of *Pomc* transcripts and α-MSH are increased under feeding conditions and decreased under fasting conditions (Schwartz *et al*. 1997). The i.c.v. administration of α-MSH or its delivery into the PVN suppresses food intake and

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reduces body weight (Poggioli *et al*. 1986, Wirth *et al*. 2001). Genetic manipulation of the *Pomc* gene leading to the overexpression of α-MSH has been shown to cause anti-obesity effects in genetic and diet-induced obesity (DIO) models (Mizuno *et al*. 2003, Savontaus *et al*. 2004, Lee *et al*. 2007). A key role for POMC in whole-body energy homeostasis is evident, as mice lacking *Pomc*, melanocortin peptides, or POMC neurons develop obesity (Yaswen *et al*. 1999, Gropp *et al*. 2005, Xu *et al*. 2005*a*, Smart *et al*. 2006). Furthermore, mutations in the *POMC* gene have been reported to be associated with morbid obesity in humans (Krude *et al*. 1998, Lee *et al*. 2006). GABAergic and glutamatergic subpopulations of POMC neurons have been described, although their functional roles are unclear (Mercer *et al*. 2013).

Paraventricular nucleus The PVN is located in the anterior hypothalamus, just above the third ventricle, and expresses high levels of MC3R/MC4R. It receives innervation not only from the AgRP and POMC neurons of the ARC but also from extrahypothalamic regions such as the nucleus of the tractus solitarius (NTS). The PVN is an important integration site involved in whole-body energy homeostasis, as shown by the diverse afferent inputs and its high sensitivity to the administration of endogenous neuropeptides involved in the regulation of food intake such as NPY, AGRP, and α-MSH, among others (Stanley *et al*. 1986, Kim *et al*. 2000). Part of these effects are mediated by a subset of neurons that express thyrotropin-releasing hormone (TRH), which are activated by α-MSH and inhibited by AGRP (Fekete *et al*. 2000, 2004). Another relevant subset of neurons express corticotrophin-releasing hormone (CRH), which are directly involved in the control of energy balance through AGRP innervation or indirectly through the regulation of adrenal glucocorticoids controlling the expression of POMC (Richard & Baraboi 2004).

Lateral hypothalamus area The LHA plays a critical role in the mediation of orexigenic responses, a function that can be significantly attributed to orexin and melanin-concentrating hormone (MCH) neurons. Orexin neurons produce orexin A and orexin B from prepro-orexin, the expression of which is increased under fasting conditions (Sakurai *et al*. 1998). The central administration of orexins not only increases food intake (Sakurai *et al*. 1998, Dube *et al*. 1999), but also promotes behavioral responses to food reward and increases arousal (Cason *et al*. 2010). Orexin neurons project not only within the LHA, ARC, PVN, and NTS, but also into other regions involved in additional physiological functions such as body temperature and wakefulness control, among others (Peyron *et al*. 1998). Similarly, fasting enhances the expression of *Mch* (*Pmch*) mRNA and its i.c.v. administration or genetic overexpression causes an orexigenic output (Qu *et al*. 1996, Ludwig *et al*. 2001). Conversely, mice with reduced MCH tone or disruption of the MCH1 receptor are lean (Marsh *et al*. 2002).

Dorsomedial nucleus The DMN is involved in a range of physiological processes, including appetite, thermoregulation, stress, and circadian rhythms. It receives projections from most of the hypothalamic nuclei, especially the ARC, and sends projections into the PVN and LHA. A number of neuropeptides (such as NPY and CRH) as well as receptors for peptides involved in the control of appetite and energy balance are expressed within the DMN. Increased expression of NPY in the DMN has been reported in several rodent models of obesity (Guan *et al*. 1998, Bi *et al*. 2001), and it may play a significant role in the regulation of thermogenesis and the development of DIO (Chao *et al*. 2011).

Ventromedial nucleus The AgRP and POMC neurons of the ARC project into the VMN. In turn, VMN neurons project into hypothalamic and extrahypothalamic areas such as the brainstem (Cheung *et al*. 2013). Laser-microdissection studies have identified a number of VMN-enriched genes (Segal *et al*. 2005), including steroidogenic factor 1 (*Sf1* (*Nr5a1*)), which has been directly implicated in the development of the VMN (Parker *et al*. 2002, Davis *et al*. 2004). SF1-expressing neurons play significant roles in the control of energy balance, as demonstrated by the metabolic phenotypes of conditional KO mice (Bingham *et al*. 2008, Zhang *et al*. 2008, Kim *et al*. 2011). Another abundantly expressed protein in the VMN is the brain-derived neurotrophic factor (BDNF). The lack of *BDNF* or its receptor (*TRKB* (*NTRK2*)) leads to hyperphagia and obesity in humans and mice (Lyons *et al*. 1999, Yeo *et al*. 2004). In contrast, the central or peripheral administration of BDNF results in the loss of body weight and reduction in food intake through MC4R signaling (Xu *et al*. 2003). The VMN also plays a key role in the regulation of thermogenesis (Lopez *et al*. 2010, Kim *et al*. 2011, Martinez de Morentin *et al*. 2012, Whittle *et al*. 2012).

The brainstem

Brainstem neurons make key contributions to the control of energy balance by processing energy status information

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at four different levels: i) by sensing circulating metabolites and hormones released by peripheral organs; ii) by receiving vagal inputs from the gastrointestinal (GI) tract; iii) by receiving neuronal inputs from midbrain and forebrain nuclei that also detect and integrate energy-related signals; and iv) by projecting into local brainstem circuits and other regions of the brain to provide information that will be integrated by these neurons to control energy balance. Within the brainstem, the dorsal vagal complex (DVC) is a key module for the integration of energy-related cues by relying peripheral signals through vagal afferents and projecting into the hypothalamus and other relevant areas. The DVC comprises the dorsal motor nucleus of the vagus, the NTS, and the area postrema (AP), which has an incomplete BBB and therefore it is accessible to peripheral signals.

The brainstem is constituted by heterogeneous populations of neurons, with distinct biophysical and neurochemical properties, that express appetite-modulatory neuropeptides such as tyrosine hydroxylase (TH), proglucagon, CART, GABA, NPY, BDNF, and POMC, among others. These neurons also express a variety of receptors mediating the effects of some of the aforementioned neuropeptides, indicating the existence of local circuits that contribute to the regulation of ingestive behaviors. In addition, receptors for a number of circulating hormones such as leptin, ghrelin, glucagon-like peptide 1 (GLP1), and cholecystokinin (CCK) have been described in brainstem neurons or in vagal afferent projections to brainstem areas.

Vagal signaling from the GI tract is an important afferent to the NTS, conveying information about luminal distension, nutritional content, and locally produced peptides via glutamate neurotransmission (Travagli *et al.* 2006). This vagal sensory and hormonal information will be assimilated by second-order NTS neurons that project into the hypothalamus and other basal forebrain areas to elaborate precise outputs. The significance of the vagus nerve transmission has been demonstrated through a number of manipulations to eliminate or enhance its activity. For example, chronic or acute vagus nerve stimulation in rats leads to a reduction in body weight and food intake, indicating that direct vagal afferent interventions influence feeding behavior (Krolczyk *et al.* 2001, Gil *et al.* 2011). Vagal signaling also plays important roles in the regulation of meal size and duration (Schwartz *et al.* 1999).

The NTS receives inputs from descending projections from the hypothalamus. In particular, ARC POMC neurons project into the NTS, where high expression levels of MC4R have been reported (Kishi *et al.* 2003). In addition to the release of α-MSH from ARC POMC neurons, the NTS also receives melanocortin agonist signals from a local population of ~300 POMC neurons (around 10% of the total number of POMC neurons; Palkovits & Eskay 1987). Recent pharmacogenetic studies have shown different functions and time scale effects of ARC and NTS POMC neurons on food intake and metabolism (Zhan *et al.* 2013). The importance of this neuronal circuit is further demonstrated by hindbrain MC4R agonist delivery, which leads to a reduction in food intake and an increase in energy expenditure, whereas MC4R antagonism drives the opposite effect (Williams *et al.* 2000, Skibicka & Grill 2009*b*). MC4Rs in the NTS seem to mediate not only the satiation effects of CCK (Fan *et al.* 2004), but also the anorexigenic effects of hypothalamic and brainstem leptin signaling (Skibicka & Grill 2009*a*, Zheng *et al.* 2010).

The NTS also receives descending projections from orexin and MCH neurons located in the LHA (Ciriello *et al.* 2003), and the delivery of orexin A into the hindbrain increases food intake (Parise *et al.* 2011). The orexigenic nature of the LHA and the anatomical connection with the NTS indicated that this system may serve as a mechanism to limit the satiety signals from the GI tract.

Another hypothalamic nucleus sending projections into the NTS is the PVN (Sawchenko & Swanson 1982, Luiten *et al.* 1985). The PVN–brainstem pathway plays a significant role in the regulation of energy balance, as contralateral disruption of PVN output and NTS input causes hyperphagic obesity (Kirchgessner & Sclafani 1988). Different areas of the brainstem show TRH-positive fibers, and evidence indicates that TRH is involved in the brainstem regulation of energy homeostasis by integrating endocrine and vagal–sympathetic responses (Ao *et al.* 2006, Zhao *et al.* 2013).

Hormonal signals involved in energy homeostasis control

Peripheral adiposity signals: leptin and insulin

The discovery of leptin, the product of the *Ob* gene, in 1994 (Zhang *et al.* 1994) opened a new dimension in the field of the central regulation of energy balance. Leptin is an anorexigenic adipose tissue-derived hormone that circulates in proportion to fat mass (Considine *et al.* 1996). It reaches the CNS through a saturable transport system and conveys information about the energy status of the organism. There are multiple leptin receptor (LEPR) isoforms, with the long form (LEPRb) being essential for

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the effects of leptin. The lack of leptin or *LEPRb* in both rodents and humans causes a phenotype characterized by hyperphagia, reduced energy expenditure, and severe obesity (Halaas *et al*. 1995, Chen *et al*. 1996, Montague *et al*. 1997, Clement *et al*. 1998). Most obese patients exhibit a state of leptin resistance, which is the inability of high circulating leptin levels to exert central anorexigenic actions, which precludes the use of leptin as a therapeutical approach.

LEPRb is highly expressed in different hypothalamic nuclei and other CNS regions involved in the control of energy balance (Elmquist *et al*. 1998). In the ARC, the POMC, and AgRP neurons are the direct targets of leptin (Cheung *et al*. 1997, Elias *et al*. 1999, Cowley *et al*. 2001). The ablation of LEPRb in POMC neurons, AgRP neurons, or both populations of neurons causes increased body weight, emphasizing the importance of leptin signaling (Table 1). However, the magnitude of these changes is smaller than that observed in mice globally lacking *Lepr*, indicating the existence of additional subsets of neurons mediating the effects of leptin on food intake and body weight. Leptin binds to LEPRb and activates JAK2, which, in turn, phosphorylates several tyrosine residues on the intracellular domain of the LEPRb. This results in the activation, dimerization, and nuclear translocation of STAT3 (Robertson *et al*. 2008). In the nucleus, STAT3 enhances *Pomc* gene expression and inhibits *Agrp* gene expression (Munzberg *et al*. 2003, Kitamura *et al*. 2006). Accordingly, *Stat3* deficiency in POMC neurons results in overweight and *Pomc* gene transcriptional defects in females (Table 1). This signaling cascade is negatively regulated by the suppressor of cytokine signaling 3 (SOCS3), the expression of which is also regulated by STAT3 and protein tyrosine phosphatase 1B (PTP1B) (Robertson *et al*. 2008). Consistent with this, deletion of either *Socs3* or *Ptp1b* (*Ptpn1*) in POMC neurons leads to reduced adiposity, improved leptin sensitivity, and increased energy expenditure under HFD conditions (Table 1). In addition, leptin also activates the phosphatidylinositol-3-kinase (PI3K) pathway. A variety of genetic mouse models targeting the catalytic or regulatory subunits of PI3K in specific subsets of neurons have been reported with divergent results (Table 1). Overall, these studies indicate that PI3K is required for leptin-mediated regulation of energy balance and that, contrary to the prevailing view, the catalytic p110β subunit in ARC neurons may play a more prominent role than p110α. PI3K generates phosphatidylinositol-3,4,5-triphosphate (PIP_3) and activates downstream targets such as phosphoinositide-dependent kinase 1 (PDK1) and AKT (also known as protein kinase B), which consecutively phosphorylates the transcription factor forkhead box protein O1 (FOXO1). Upon phosphorylation, FOXO1 is excluded from the nucleus, allowing STAT3 to bind to *Pomc* and *Agrp* promoters, thereby stimulating and inhibiting respectively the expression of these neuropeptides (Kitamura *et al*. 2006). These findings are consistent with the effects of genetic manipulations *in vivo* (Table 1). PI3K signaling is counterbalanced by phosphatase and tensin homolog (PTEN), which specifically dephosphorylates PIP_3. The loss of *Pten* in POMC neurons results in increased PIP_3 signaling and diet-sensitive obesity via K_{ATP} channel modulation, suggesting a role for the PI3K pathway in the regulation of the activity of this channel (Table 1). Overall, leptin stimulates *Pomc* transcription, depolarizes POMC neurons, and also increases α-MSH processing and secretion (Cowley *et al*. 2001, Munzberg *et al*. 2003, Guo *et al*. 2004) while attenuating the expression and release of orexigenic NPY and AGRP neuropeptides (Stephens *et al*. 1995, Mizuno & Mobbs 1999).

Insulin, produced by pancreatic β-cells, has traditionally been associated with glucose metabolism, but compelling evidence indicates that insulin also acts as an anorectic signal within the CNS. Glucose-induced insulin is secreted into the bloodstream in proportion to fat stores (Bagdade *et al*. 1967) and enters the brain through a saturable transport mechanism (Baura *et al*. 1993). The i.c.v. or intrahypothalamic administration of insulin to primates and rodents reduces food intake (Woods *et al*. 1979, McGowan *et al*. 1993, Air *et al*. 2002). Insulin receptor (IR (INSR)), as well as its downstream signaling machinery, is expressed in hypothalamic areas involved in the control of appetite (Havrankova *et al*. 1978, Corp *et al*. 1986) and colocalizes with AgRP and POMC neurons (Benoit *et al*. 2002). Surprisingly, the loss of *Insr* in either POMC or AgRP neurons does not lead to alterations in energy balance (Table 1), although hepatic glucose production defects have been observed in mice lacking *Ir* in AgRP neurons (Konner *et al*. 2007). Neuron-specific IR reconstitution in L1 mice (which have >90% reduction of IR levels in the ARC) confirmed that insulin signaling in AgRP and POMC neurons controls glucose metabolism and energy expenditure respectively (Table 1). Insulin binding to IR leads to the autophosphorylation of the receptor and the consequent recruitment of IRS proteins, which converge with the leptin pathway at the PI3K level (Xu *et al*. 2005*b*). Negative regulators of the LEPR, such as SOCS3 and PTP1B, also directly inhibit the IR and its signaling cascade acting on IRS1. The activation of the

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Table 1 Summary of relevant genetic mouse models used in the analysis of leptin and insulin signaling pathways in POMC and AgRP neurons

Genetic manipulation	Neuronal cell type	BW	Adiposity	Food intake	Energy expenditure	Diet	Other features	References
Lepr deletion	POMC	+	+	=	=	Chow	Altered neuropeptide expression	Balthasar *et al.* (2004)
Lepr deletion	AgRP	+	+	=	=	Chow	Reduced locomotor activity	van de Wall *et al.* (2008)
Lepr deletion	POMC and AgRP	+	+	Transient +	−	Chow	Increased respiratory exchange ratio	van de Wall *et al.* (2008)
Ir deletion	POMC	=	=	=	ND	Chow and HFD	–	Konner *et al.* (2007)
Ir deletion	AgRP	=	=	=	ND	Chow and HFD	Enhanced hepatic glucose production	Konner *et al.* (2007)
Ir re-expression in L1 mice	POMC	−	=	+	+	Chow	Insulin resistance	Lin *et al.* (2010)
Ir re-expression in L1 mice	AgRP	−	=	=	+	Chow	Rescued hepatic glucose production	Lin *et al.* (2010)
Lepr and *Ir* deletion	POMC	+	=	=	−	Chow	Insulin resistance and reduced fertility in females	Hill *et al.* (2010)
Irs2 deletion	POMC	=	=	=	=	Chow	Normal insulin and leptin levels	Choudhury *et al.* (2005)
Ptp1b deletion	POMC	−	−	=	+	HFD	Improved leptin sensitivity	Banno *et al.* (2010)
Stat3 deletion	POMC	+	+	+	ND	Chow	Normal phenotype in male mice	Xu *et al.* (2007)
Stat3 deletion	AgRP	+	+	+	ND	Chow	Hyporesponsive to leptin	Gong *et al.* (2008)
Stat3 constitutive active form	POMC	+	+	+	ND	Chow	No additional effect on HFD administration	Ernst *et al.* (2009)
Stat3 constitutive active form	AgRP	−	−	=	+	Chow and HFD	Increased locomotor activity	Mesaros *et al.* (2008)
Pdk1 deletion	POMC	+	+	+	=	Chow	Decreased *Pomc* gene expression	Iskandar *et al.* (2010)
Pdk1 deletion	AgRP	−	−	−	=	Chow	Rescued by dominant negative Foxo1	Cao *et al.* (2011)
Pdk1 deletion	POMC	Transient +	Transient +	Transient +	ND	Chow and HFD	Rescued by dominant negative Foxo1	Belgardt *et al.* (2008)
Foxo1 deletion	POMC	−	−	−	=	Chow	Increased *Cpe* expression and α-MSH levels	Plum *et al.* (2009)
Foxo1 constitutive active form	POMC	+ (Females)	+ (Females)	+ (Females)	=	Chow	Decreased *Pomc* gene expression	Iskandar *et al.* (2010)
Foxo1 deletion	AgRP	=	−	−	=	Chow	Resistant to HFD	Ren *et al.* (2012)
Socs3 deletion	POMC	−	ND	=	+	HFD	No body weight phenotype on chow diet	Kievit *et al.* (2006)
Socs3 overexpression	POMC	+	+	=	−	Chow	Leptin resistance	Reed *et al.* (2010)
Socs3 overexpression	AgRP	=	=	+	+	Chow	Altered glucose metabolism	Olofsson *et al.* (2013)
Pten deletion	POMC	+	+	+	=	Chow	Gender dimorphism on HFD administration	Plum *et al.* (2009)

Table 1 *Continued*

Genetic manipulation	Neuronal cell type	BW	Adiposity	Food intake	Energy expenditure	Diet	Other features	References
p85 (*Pik3r1*) deletion	POMC	=	ND	ND	ND	Chow	Gender dimorphism on HFD administration	Hill *et al.* (2009)
p110α (*Pik3ca*) deletion	POMC	+	+	=	– (Females)	Chow	Sensitive to HFD	Hill *et al.* (2009)
P110α deletion	POMC	=	=	=	=	Chow	Sensitive to HFD	Al-Qassab *et al.* (2009)
p110α deletion	AgRP	=	=	=	=	Chow and HFD	Blunted insulin-induced depolarization	Al-Qassab *et al.* (2009)
p110β (*Pik3cb*) deletion	POMC	=	+	+	=	Chow	Sensitive to HFD	Al-Qassab *et al.* (2009)
p110β deletion	AgRP	–	–	–	=	Chow and HFD	Blunted insulin-induced depolarization	Al-Qassab *et al.* (2009)
Ampkα2 (*Prkaa1*) deletion	POMC	+	+	+ After fast	–	Chow and HFD	Neurons insensitive to glucose changes	Claret *et al.* (2007)
Ampkα2 deletion	AgRP	–	=	=	=	Chow	Neurons insensitive to glucose changes	Claret *et al.* (2007)

ND, not determined.

IR signaling pathway results in reduced expression of NPY and increased levels of POMC in the ARC, thus stimulating an anorexigenic effect (Schwartz *et al.* 1992, Sipols *et al.* 1995, Benoit *et al.* 2002).

Leptin and insulin also regulate the activity of AMPK, an evolutionarily conserved cellular and organismal energy sensor that plays a central role in the hypothalamic regulation of energy homeostasis (Minokoshi *et al.* 2004, Claret *et al.* 2007). In particular, both hormones inhibit AMPK and its downstream targets in the hypothalamus (Minokoshi *et al.* 2004). A recent study has reported that leptin-mediated inhibition of AMPK is achieved through phosphorylation on serine^{491} by mTOR/p70S6K, an event that is necessary for the action of leptin on food intake and body weight (Dagon *et al.* 2012).

The molecular significance and detailed mechanisms of the different components of the aforementioned signaling pathways have become better understood, thanks to the advent of the Cre/Lox technology. Table 1 summarizes the phenotypes of several conditional mouse models that provided valuable information in this regard.

GI hormones

Ghrelin is a 28-amino acid acylated hormone, mainly produced by the stomach, which exerts its biological actions on energy balance through the growth hormone secretagogue receptor (GHSR; Kojima *et al.* 1999, Sun *et al.* 2004). Circulating ghrelin levels are increased under fasting conditions and reduced after refeeding (Tschop *et al.* 2000). The central and peripheral administration of ghrelin in rodents has been shown to robustly promote feed intake, adiposity, and body weight gain (Tschop *et al.* 2000, Nakazato *et al.* 2001). Likewise, ghrelin also enhances appetite in humans (Wren *et al.* 2001). GHSR is expressed in AgRP neurons of the ARC (Willesen *et al.* 1999), and this population of neurons is essential for the mediation of the orexigenic effects of ghrelin (Chen *et al.* 2004). Ghrelin is able to stimulate the transcription of *Npy* and *Agrp*, and it also increases the number of stimulatory synapses on AgRP neurons while increasing the number of inhibitory synapses on POMC neurons (Kamegai *et al.* 2001, Nakazato *et al.* 2001, Cowley *et al.* 2003). However, neuronal activation and positive energy balance have also been reported after ghrelin administration in the PVN, LHA, and hindbrain and in the mesolimbic reward pathway (Faulconbridge *et al.* 2003, Naleid *et al.* 2005).

Peptide tyrosine tyrosine (PYY) is mainly released from the L-cells of the intestinal epithelium in response to nutrient ingestion (Tatemoto & Mutt 1980,

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Adrian *et al*. 1985). Circulating PYY levels are proportional to calorie intake and are reduced under fasting conditions (Adrian *et al*. 1985). Two endogenous forms, $PYY_{1–36}$ and $PYY_{3–36}$, are synthesized and secreted. The latter form is the most abundant in the bloodstream and exerts a direct action in the ARC. This has been demonstrated by peripheral and intra-ARC administration of $PYY_{3–36}$, which increases neuronal activity in this region and reduces appetite and body weight in a dose-dependent manner (Batterham *et al*. 2002, Challis *et al*. 2003). These anorexigenic effects are mediated via the inhibition of ARC Y2 receptors, as demonstrated by pharmacological (Abbott *et al*. 2005, Scott *et al*. 2005) and genetic (Batterham *et al*. 2002) studies, which eventually leads to increased α-MSH and reduced NPY release (Batterham *et al*. 2002). The effects of $PYY_{3–36}$ in the brainstem and the vagal–brainstem circuit have also been confirmed, as the peripheral delivery of this peptide has been shown to increase neuronal activity in NTS and AP neurons and stimulate vagal afferent firing (Koda *et al*. 2005, Blevins *et al*. 2008). Consistent with a role for PYY in the regulation of appetite and body weight, transgenic mice globally lacking or overexpressing *Pyy* exhibit opposite alterations in energy balance control (Batterham *et al*. 2006, Boey *et al*. 2008).

GLP1, the cleavage product of proglucagon in the intestine and brain, is mainly secreted from intestinal L-cells. Similar to PYY, circulating GLP1 levels are high following a meal and are low under fasting conditions. This hormone exerts a strong incretin effect, via the GLP1 receptor (GLP1R) expressed in pancreatic islets, enhancing insulin secretion after carbohydrate ingestion (Kreymann *et al*. 1987). GLP1R is also expressed in key CNS areas involved in the control of energy balance, such as the hypothalamus and brainstem (Merchenthaler *et al*. 1999). A number of studies have shown that the central or site-specific administration of GLP1 or GLP1 analogs inhibits food intake in rodents (Tang-Christensen *et al*. 1996, Turton *et al*. 1996, McMahon & Wellman 1998, Hayes *et al*. 2008). Interestingly, neurons expressing the proglucagon gene are present in the NTS, suggesting the existence of a local circuit involved in the control of appetite (Merchenthaler *et al*. 1999). In fact, recent studies have provided evidence for a dual (peripheral and central) role of GLP1 in the suppression of appetite mediated by local vagal afferents and a gut–brain feedback mechanism (Barrera *et al*. 2011).

CCK is postprandially secreted from I-cells from the small intestine and its systemic delivery suppresses food intake in both animal models and humans (Gibbs *et al*. 1973, Gibbs & Smith 1977, Kissileff *et al*. 1981). CCK1 and CCK2 receptors are expressed in the brainstem and hypothalamus, but the anorectic effects of CCK are critically mediated by vagal sensory neurons that project into the NTS (Moran *et al*. 1997). Interestingly, NTS POMC neurons are activated by CCK and brainstem MC4R signaling is required for CCK-induced suppression of appetite (Fan *et al*. 2004). It has also been reported that ghrelin attenuates and leptin synergistically potentiates the effects of CCK on appetite (Barrachina *et al*. 1997, Lee *et al*. 2011).

Neural circuits regulating homeostatic energy balance

Certain physiological conditions, such as the prandial state, are associated with notable changes in the circulating concentration of metabolites and hormones involved in the regulation of whole-body energy homeostasis. For example, in a postabsorptive situation, circulating cues of energetic surfeit (leptin, insulin, GLP1, PYY, and glucose) are elevated, while cues of energetic deficit (ghrelin) are reduced. The opposite is true under fasting conditions. These hormones act in concert to engage specific neuronal circuits in different brain regions, including the hypothalamus and brainstem, establishing reciprocal and dynamic interactions to restore systemic energy balance. In this section, we summarize the main circuits and the neuronal responses engaged by leptin and ghrelin, as prototypical examples of anorexigenic and orexigenic signals respectively.

ARC neuronal circuits: POMC, AgRP, and RIPCre neurons

Melanocortin peptides and NPY are two basic components of a critical hypothalamic circuit involved in the convergence and integration of nutritional and hormonal cues aimed at regulating organismal energy balance. In the ARC, the POMC, and AgRP neurons are located in proximity to each other and project in parallel into similar brain areas expressing MCRs. Both POMC and AgRP neurons are able to sense a number of peripheral (leptin, insulin, and ghrelin) and central (NPY, GABA, serotonin, and melanocortin) signals, which are able to acutely modulate their electrical activity influencing the release of neuropeptides and neurotransmitters to ultimately regulate appetite, energy expenditure, and metabolism.

In general terms, POMC (anorexigenic) and AgRP (orexigenic) neurons have opposite physiological functions, which are largely the consequence of the

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contrasting actions of α-MSH and AGRP peptides on MCRs: while α-MSH is an endogenous MCR agonist, AGRP is an inverse agonist (Haskell-Luevano & Monck 2001, Nijenhuis *et al*. 2001, Tolle & Low 2008). Indeed, substantial experimental evidence indicates that the agonism of MCRs attenuates appetite and enhances energy expenditure, whereas their antagonism has essentially the opposite effects (Fan *et al*. 1997, Harrold *et al*. 1999, Hwa *et al*. 2001). This is consistent with data showing that the loss of or mutations in *MC3R* and *MC4R* genes cause obesity both in rodents and in humans (Huszar *et al*. 1997, Butler *et al*. 2000, Farooqi 2008). In addition to the inhibition of MCR signaling, the orexigenic actions of AgRP neurons are also mediated by the release of NPY and GABA.

The anorexigenic effects of leptin are basically achieved by repressing AgRP neurons and activating POMC neurons (Fig. 1A). Leptin enhances *Pomc* gene expression and processing into α-MSH (Schwartz *et al*. 1997, Thornton *et al*. 1997, Mizuno *et al*. 1998). Electrophysiological studies have demonstrated that locally applied leptin is able to depolarize (excite) POMC neurons (Cowley *et al*. 2001, Claret *et al*. 2007, 2011, Hill *et al*. 2008, Al-Qassab *et al*. 2009, Qiu *et al*. 2010) probably through TRPC channels (Qiu *et al*. 2010). In contrast, leptin inhibits the transcription of *Npy* and *Agrp* genes in the hypothalamus (Stephens *et al*. 1995, Schwartz *et al*. 1996, Mizuno & Mobbs 1999). Electrophysiological recordings have shown that leptin decreases the GABAergic-mediated tone induced by AgRP neurons onto neighboring POMC neurons, resulting in the disinhibition of POMC neuron activity (Cowley *et al*. 2001). The ability of leptin to directly hyperpolarize (inhibit) AgRP neurons is controversial (Cowley *et al*. 2001, Claret *et al*. 2007, Al-Qassab *et al*. 2009), but studies in rats have reported leptin-mediated inhibition of identified NPY neurons (van den Top *et al*. 2004). In addition, leptin also acts directly on presynaptic GABAergic neurons that do not express AGRP, reducing the inhibitory input to postsynaptic POMC neurons, thus further contributing to the maintenance of the anorexigenic actions mediated by this hormone (Fig. 1A; Vong *et al*. 2011).

On the other hand, under conditions of negative energy balance, circulating ghrelin levels are increased. The actions of ghrelin on food intake and energy balance are mediated by AgRP neurons, as mice lacking *Agrp* and *Npy* are insensitive to the orexigenic effects of external ghrelin (Chen *et al*. 2004, Luquet *et al*. 2007). In line with this, ghrelin increases the expression of *Npy* and *Agrp* transcripts (Kamegai *et al*. 2001, Nakazato *et al*. 2001) and depolarizes AgRP neurons while increasing the number of GABAergic inhibitory synapses on POMC neurons (Fig. 1B) (Cowley *et al*. 2003, van den Pol *et al*. 2009, Yang *et al*. 2011, Atasoy *et al*. 2012). The importance of these GABAergic stimuli in the control of energy balance has been substantially demonstrated (Horvath *et al*. 1997, Wu *et al*. 2009, 2012, Wu & Palmiter 2011), and conditional deletion of the vesicular GABA transporter in AgRP neurons blunts the inhibitory tone onto postsynaptic POMC neurons, leading to an enhanced melanocortigenic output and a lean phenotype (Tong *et al*. 2008). Moreover, AGRP and NPY directly hyperpolarize POMC neurons and decrease the production and release of α-MSH, further inhibiting the activity of this population of neurons (Roseberry *et al*. 2004, Smith *et al*. 2007, Cyr *et al*. 2013). Thus, AgRP neurons are able to negatively modulate the anorexigenic effects of POMC neurons by direct (GABAergic synapsis) and indirect (MCR antagonism) mechanisms (Fig. 1B).

In addition to changes in neuropeptide release, leptin and ghrelin also exert rapid and reversible effects on synaptic connections onto POMC and AgRP neurons. Seminal studies carried out at the Horvath laboratory have provided the first evidence for synaptic plasticity in hypothalamic energy balance circuits and established the basis for a new mechanism by which these hormones dynamically regulate circuit responsiveness to control energy homeostasis (Pinto *et al*. 2004). The role of synaptic remodeling in neuronal circuits regulating metabolism has recently been reviewed in detail (Zeltser *et al*. 2012, Dietrich & Horvath 2013).

A novel subpopulation of ARC neurons involved in the control of energy balance (defined by virtue of Cre-mediated expression of rat insulin II promoter-*Cre* transgene and called RIPCre neurons) has recently been described. Comparative electrophysiological and histological studies indicate that RIPCre neurons constitute a distinct population from POMC or AgRP neurons (Choudhury *et al*. 2005). However, close apposition of these neuronal subsets suggests that RIPCre neurons may be the targets of POMC and/or AgRP neurons. Indeed, bath application of a melanocortin agonist has been found to cause direct long-lasting depolarization and increased firing in ARC RIPCre neurons (Choudhury *et al*. 2005). Interestingly, insulin has also been found to depolarize these neurons, while leptin has been found to not cause any electrophysiological effect (Choudhury *et al*. 2005).

Although a number of mouse genetic studies indicate that ARC RIPCre neurons regulate systemic energy balance (Cui *et al*. 2004, Choudhury *et al*. 2005), this interpretation

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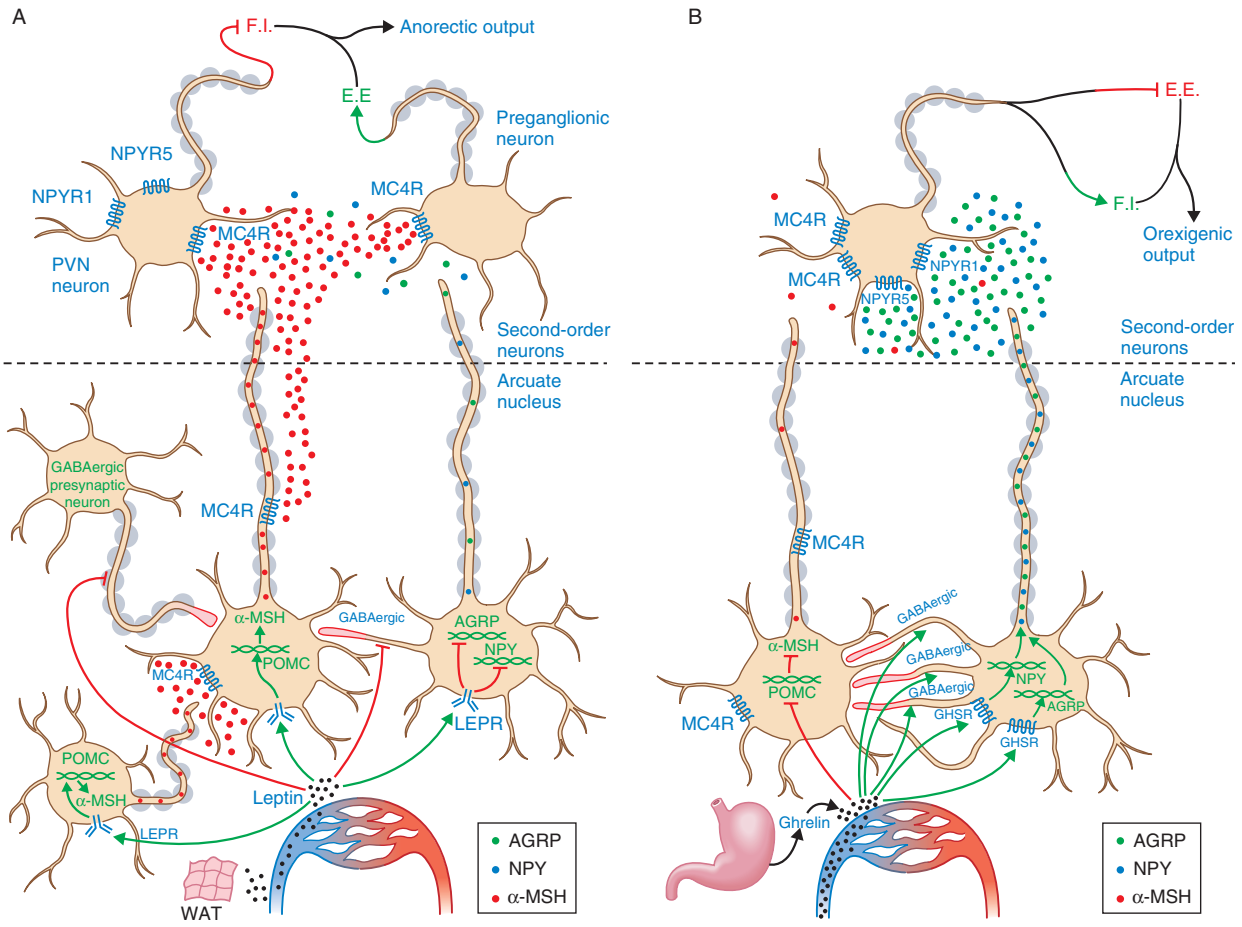

Figure 1

Schematic representation of the main neuronal circuits engaged by leptin and ghrelin. (A) Leptin is released in proportion to fat stores and stimulates the activity of anorexigenic POMC neurons in the ARC while inhibiting neighboring AgRP neurons. This results in increased release of α-MSH and the activation of downstream second-order neurons expressing MC4R in hypothalamic and extrahypothalamic regions. POMC neurons also express MC4R, indicating the existence of an autoregulatory mechanism induced by α-MSH. Leptin also acts on GABAergic presynaptic neurons attenuating its inhibitory effect on POMC neurons. Overall, these effects result in reduced food intake and increased energy expenditure. (B) Ghrelin exerts its orexigenic effects through AgRP neurons. Ghrelin increases inhibitory GABAergic projections onto POMC neurons and enhances the expression and release of NPY and AGRP. In the PVN, AGRP acts as a MC4R inverse agonist, while NPY binds to Y1 and Y5 receptors. Collectively, these events lead to increased orexigenic output. Red arrows and synapses, inhibitory effect and green arrows, activation effect. WAT, white adipose tissue.

is called into question by the fact that the RIPcre transgene is also expressed in other regions of the brain and pancreatic β-cells. However, recent data show that the acute and selective ablation of ARC RIPCre neurons leads to hypophagia, reduced food intake, and adiposity through compensatory increase in the number of anorexigenic neurons in the PVN (Rother *et al.* 2012). Consistent with the anorexigenic nature of RIPCre neurons, a combination of genetic and pharmacogenetic approaches has shown that the synaptic release of GABA, but not of glutamate, from this subset of neurons increases the thermogenic function of BAT without affecting food intake (Kong *et al.* 2012). The effects of leptin on RIPCre neurons are complex, as suggested by heterogeneous electrophysiological recordings demonstrating subsets of neurons being depolarized, hyperpolarized, or silent (Choudhury *et al.* 2005, Kong *et al.* 2012). Nevertheless, the ability of leptin to increase energy expenditure is impaired in mice lacking vesicular GABA transporter in RIPCre neurons, indicating a functional effect of this hormone on these neurons (Kong *et al.* 2012).

Taken together, current evidence indicates that a local ARC circuit constituted by the 'first-order' POMC, AgRP, and RIPCre neurons plays a key role in the integration of humoral signals reporting on energy conditions. This is achieved by a sophisticated and multilevel

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organizational structure that allows the accurate regulation of orexigenic and anorexigenic outputs through direct and indirect mechanisms.

Downstream neurocircuitry engaged by hypothalamic neuron activity

Given that POMC and AgRP neurons are the sole source of MCR ligands in the brain, a fine balance between α-MSH and AGRP is necessary to precisely regulate their mediated physiological outputs on MC4Rs in target areas. These receptors are localized in many nuclei involved in the regulation of energy balance where POMC and AgRP neurons send axon projections. MC4Rs are G-protein-coupled receptors that stimulate adenylyl cyclase, thereby increasing intracellular cAMP levels (Florijn *et al.* 1993). A series of elegant studies using a cell-specific MC4R re-expression strategy indicate that MC4Rs in the PVN are mainly involved in the control of food intake (Balthasar *et al.* 2005), while MC4Rs in autonomic preganglionic neurons regulate energy expenditure and hepatic glucose production (Rossi *et al.* 2011) (Fig. 1A). Furthermore, and contrary to the prevailing view, a recent report has shown that POMC neurons also express MC4Rs that contribute to the regulation of body weight and composition through changes in both feeding behavior and energy expenditure (do Carmo *et al.* 2013). This autoregulatory mechanism, induced by α-MSH released from the same cell and/or neighboring POMC neurons, could represent an additional layer of regulation within a widely segregated network of melanocortin receptors involved in the regulation of homeostatic (appetite) and autonomic (thermogenesis, hepatic metabolism, and insulin release) functions (Fig. 1A).

NPY receptors are Gi/o-protein-coupled receptors that reduce cAMP production, leading to the activation of G-protein-gated inwardly rectifying K^+ (GIRK) channels and inhibition of voltage-dependent Ca^{2+} channels (Sohn *et al.* 2013). The precise roles of NPY receptors and their contribution to the mediation of the orexigenic effects of NPY have been difficult to delineate due to the paradoxical phenotypes of receptor KO mouse models. This is probably the consequence of receptor redundancies and compensatory mechanisms exhibited after the application of germline deletion strategies. Despite these limitations, pharmacological and genetic studies indicate that the orexigenic actions of NPY are mediated by postsynaptic Y1 and Y5 within the PVN (Nguyen *et al.* 2012, Sohn *et al.* 2013; Fig. 1B). Notably NPY from ARC neurons acts through PVN Y1, resulting in a functional inhibition of TH tonus and BAT thermogenesis (Shi *et al.* 2013). Furthermore, NPY may also decrease pro-TRH transcription and proconvertase 2-mediated pro-TRH processing in the PVN through Y1/Y5 receptors (Cyr *et al.* 2013). Taken together, abundant amounts of evidence suggest that the effects of ARC NPY on energy balance are principally mediated by the PVN. However, it is important to note that other sources of NPY may also play a role in the regulation of energy balance.

Correlating neuronal circuit activity with behavioral responses by pharmacogenetic and optogenetic techniques

Most of the experimental findings that have allowed researchers to outline the models suggested so far are largely the result of circumstantial evidence. However, the recent development of pharmacogenetic and optogenetic techniques has provided a way to exert temporally and spatially precise control over the activity of defined circuit elements. This permits the establishment of causal connections between circuit activity and behavioral responses (Sternson 2013).

Using an elegant combination of optogenetics and mouse genetic approaches, Aponte *et al.* (2011) have confirmed that the selective activation of AgRP neurons is sufficient to evoke voracious feeding behavior in mice, without previous training and independent of melanocortin signaling. The level of neuronal activation has been found to correlate with the magnitude, dynamics, and duration of the induced behavioral response. Furthermore, continuous photostimulation is required to maintain evoked feeding behaviour, indicating that the activation of AgRP neurons does not initiate a sustained propagating effect (Aponte *et al.* 2011). In contrast, prolonged (but not brief) optogenetic stimulation of POMC neurons has been shown to result in reduced food intake and body weight gain, which requires downstream MC4R activity (Aponte *et al.* 2011).

The behavioral effects on food intake caused by AgRP or POMC neuron activation have been further supported by studies using pharmacogenetic (designer receptors exclusively activated by designer drugs (DREADDs)) technology. Pharmacogenetic activation of AgRP neurons rapidly induces feeding and food-seeking behaviors associated with decreased energy expenditure and enhanced adiposity (Krashes *et al.* 2011). Consistent with the optogenetic data (Aponte *et al.* 2011), long-term stimulation of ARC POMC neurons is necessary to reduce appetite. Interestingly, the acute stimulation of NTS POMC neurons has been shown to generate an immediate suppression of food intake (Zhan *et al.* 2013).

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In a subsequent study, the Sternson group performed a series of experiments to determine which brain regions and cell types mediate evoked feeding behavior triggered by activated AgRP neurons. The authors used optogenetic approaches to map synaptic connections downstream of AgRP neurons and assessed their role in terms of ingestive behavior by perturbing electrical activity in presynaptic and postsynaptic neuronal types (Atasoy *et al.* 2012). Notably the authors found that ARC AgRP neurons induce evoked feeding behavior through inhibitory input onto oxytocin neurons in the PVN, while ARC POMC neurons are involved in the long-term control of appetite and energy balance (Atasoy *et al.* 2012).

Collectively, these results emphasize the previously unrecognized importance of the temporal and spatial activation of POMC and AgRP neurons. Thus, ARC AgRP and NTS POMC neurons could be involved in the regulation of acute feeding behavior while ARC POMC neurons may be involved in long-term responses. This demonstrates the existence of multiple, distinct behavioral and anatomical modules that act in synchrony to regulate whole-body energy balance. The use of these tools in the field of central control of energy balance has provided novel valuable information and has confirmed previous findings. However, it has also generated some controversial observations. Further research needs to be conducted to precisely define the importance of these factors and to reconcile these observations with previous evidence (Mercer *et al.* 2013). Nevertheless, these reports demonstrate that optogenetics and pharmacogenetics are exceptionally useful tools to study the interrelationships between synaptology, neuronal circuit activity, and behavioral outputs.

New players in energy balance control

Non-neuronal cell types: macroglia and microglia

Glial cells have traditionally been considered satellite neuronal partners with supportive and structural roles. However, in recent years, glial cells have acquired a new rank and are now regarded as active players in many physiological functions including energy balance control.

Astrocytes are star-shaped cells that are involved in a number of functions, such as metabolic support to neurons and transmitter uptake and release as well as synaptic remodeling (Sofroniew & Vinters 2010). Astrocytes express LEPR (Cheunsuang & Morris 2005, Hsuchou *et al.* 2009*b*), and modifications in circulating leptin levels alter hypothalamic astrocyte expression of structural proteins as well as glutamate and glucose transporters (Garcia-Caceres *et al.* 2011, Fuente-Martin *et al.* 2012). This may cause changes in the synaptic plasticity and excitability of surrounding neurons, leading to metabolic adaptations. In fact, HFD administration in rodents is associated with increased glial coverage of POMC neuron perikarya (Horvath *et al.* 2010). It has also been reported that DIO mice exhibit increased expression of functional astrocytic LEPR in the hypothalamic region, an effect that may play a role in the development of leptin resistance (Hsuchou *et al.* 2009*a*). Indeed, loss of astrocytic *Lepr* under HFD conditions provides partial protection against developing disturbances in neuronal leptin signaling (Jayaram *et al.* 2013).

Obesity and lipid overload induce chronic low-grade inflammation in the hypothalamus (Thaler *et al.* 2010). This is regarded as a protective effect, which is mainly promoted by microglial cells that have immunitary actions in the CNS. HFD feeding selectively and rapidly activates microglia in the hypothalamus and increases the production of proinflammatory cytokines (De Souza *et al.* 2005, Milanski *et al.* 2009, Thaler *et al.* 2012). Interestingly, it has been demonstrated that moderate physical activity reduces hypothalamic microglial activation independently of body mass (Yi *et al.* 2012). Enhanced hypothalamic microglial activation has also been reported in rodents and primates after nutritional manipulations during the prenatal or perinatal period (Grayson *et al.* 2010, Tapia-Gonzalez *et al.* 2011).

Tanycytes have recently emerged as novel modulators of the hypothalamic networks that control energy balance. They contact the cerebrospinal fluid and send processes that come into proximity with neurons into the ARC and VMN (Bolborea & Dale 2013). Although it is not known whether tanycytes are able to modulate the activity of hypothalamic neurons, several lines of evidence suggest that this particular cell type may be involved in the regulation of energy homeostasis. For example, tanycytes respond to fluctuations in glucose concentration (Frayling *et al.* 2011), express a number of genes related to energy homeostasis control (Bolborea & Dale 2013), and regulate the permeability properties of the fenestrated capillaries of the ME, which may constitute a way of modulating the access of metabolites into the ARC (Langlet *et al.* 2013). Intriguingly, tanycytes may be a novel population of adult neural stem cells in the hypothalamus. Tanycytes express stem cell markers, including nestin and SOX2 (Lee *et al.* 2012), and lineage-tracing studies have shown that they give rise to neurons *in vivo* with functional implications. While short-term HFD feeding promotes hypothalamic neurogenesis in pre-adult ages (Lee *et al.* 2012), chronic

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HFD administration causes depletion of hypothalamic neural stem cells (Li *et al*. 2012). Furthermore, the manipulation of hypothalamic neurogenesis in adult mice has also produced divergent results. Selective inhibition of ME neurogenesis in adult mice fed a HFD resulted in reduced weight gain and adiposity due to enhanced energy expenditure (Lee *et al*. 2012). By contrast, genetic IKKβ/NF-κB activation in SOX2-positive hypothalamic cells led to overeating and weight gain (Li *et al*. 2012). It is important to note that these strategies did not exclusively target tanycytes and so these metabolic effects cannot be solely attributed to this cell type. Together, these results indicate that neurogenesis after short- or long-term HFD administration may have a compensatory or detrimental effect respectively on cell fate. These differences can also be the consequence of targeting distinct tanycyte populations (Bolborea & Dale 2013).

Epigenetic mechanisms

The interplay between genetic and environmental factors (nutrition, maternal health, chemicals, lifestyle, etc.) during the prenatal or perinatal period and their influence on the development of energy balance and metabolic alterations into adulthood have recently received substantial interest. In both humans and animal models, prenatal or perinatal nutritional manipulations lead to chronic metabolic disturbances in terms of feeding behavior, energy expenditure, leptin sensitivity, and glucose homeostasis. These metabolic defects may be partially the consequence of abnormal development of appetite-regulating neuronal circuits due to perinatal programming (Contreras *et al*. 2013). Epigenetic changes have been proposed as likely candidates to mediate, at least in part, these neuronal programming events, but a limited number of studies have explored this hypothesis. The epigenetic machinery that controls chromatin dynamics includes DNA methylation, posttranslational histone modifications, and non-coding RNAs. Neonatal overfeeding in rats, which results in overweight and metabolic syndrome, is associated with the hypermethylation of the *Pomc* gene promoter (Plagemann *et al*. 2009). The extent of this DNA methylation is negatively correlated with the expression of POMC in relation to leptin and insulin levels, indicating the functionality of acquired epigenomic alterations (Plagemann *et al*. 2009). In the same overnutrition model, Plagemann *et al*. (2010) also found increased methylation of the *Insr* promoter in the hypothalamus. Similarly, epigenetic remodeling of hypothalamic genes induced by mild maternal undernutrition (Stevens *et al*. 2010, Begum *et al*. 2012) or stress (Paternain *et al*. 2012) has also been reported to be associated with altered energy balance and metabolism in experimental animal models. In humans, different methylation patterns of *POMC* and *NPY* promoter regions in leukocytes have been proposed as biomarkers to predict weight regain after an energy restriction program (Crujeiras *et al*. 2013). Collectively, this evidence supports the hypothesis that early prenatal or postnatal environmental perturbations cause chronic metabolic alterations that are partially the consequence of epigenetic changes in key genes and areas of the CNS involved in the control of energy balance. Nevertheless, further research is warranted to address the significance of these epigenetic events.

MicroRNAs (miRNAs), a class of small, non-coding RNAs that regulate gene expression at the posttranscriptional level, have recently been suggested to be involved in the hypothalamic control of energy balance. It has been demonstrated that the expression of *Dicer1*, an essential endoribonuclease for miRNA maturation, is regulated by nutrient availability and excess in the hypothalamus (Schneeberger *et al*. 2013). Furthermore, we have also shown that deletion of *Dicer1* in POMC neurons leads to an obese phenotype characterized by increased adiposity, hyperleptinemia, defective glucose metabolism, and alterations in the pituitary–adrenal axis. This phenotype is associated with a progressive POMC neuron degeneration, indicating a key role for miRNAs in the survival of this population of neurons (Greenman *et al*. 2013, Schneeberger *et al*. 2013). High-throughput sequencing studies in ARC and PVN of rats have shown a specific miRNA enrichment pattern that could be used to define a prototypic profile in these brain regions. These miRNAs include seven of the eight genes of the let-7 family, the two miR-7 genes, miR-9 gene, and 5′ copy of the three miR-30 loci (Amar *et al*. 2012). Moreover, *in situ* hybridization experiments have revealed a limited and distinct expression of miR-7a in the hypothalamus, preferentially colocalizing with AgRP neurons (Herzer *et al*. 2012). Despite these efforts in describing the miRNA transcriptome and patterns of expression in the hypothalamus, the role of specific miRNAs in particular neuronal circuits in the regulation of whole-body energy balance still remains unknown.

Concluding remarks: neuronal circuitry integration and physiological responses

As has been outlined above, organismal energy balance is regulated by many factors through complex and

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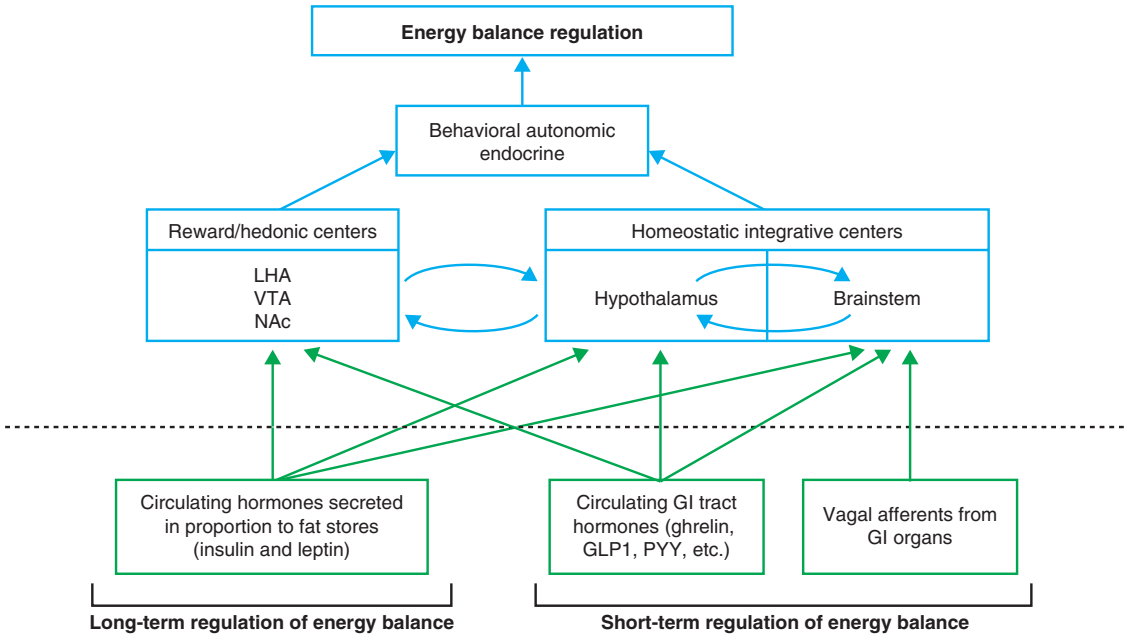

Figure 2
Schematic integration of the different levels of food intake and energy balance regulation. Food intake and energy balance are coordinately regulated by homeostatic and non-homeostatic neural mechanisms. Circulating hormones and vagus stimuli inform the CNS about whole-body nutritional and energy status. Leptin and insulin are believed to be involved in the long-term regulation of energy balance, while GI hormones and vagal afferents represent a short-term regulatory mechanism. These hormones act in concert to engage specific neuronal circuits in homeostatic and hedonic centers, establishing dynamic and complex interactions between these different brain regions to elaborate coordinated endocrine, autonomic, and behavioral responses to regulate energy balance. Sensory, emotional, and social cues also influence ingestive behaviors probably through non-homeostatic and higher brain structures. LHA, lateral hypothalamic area; VTA, ventral tegmental area; NAc, nucleus accumbens.

multi-level integration processes that involve multiple neuronal circuits. The homeostatic system is basically influenced by long-term (leptin and insulin) and short-term (GI hormones and vagal inputs) signals that act in concert to engage specific neuronal circuits in the hypothalamus and brainstem aimed at fulfilling whole-body metabolic needs. In addition to this homeostatic module, the corticolimbic and mesolimbic centers (which include the ventral tegmental area, nucleus accumbens, prefrontal cortex, hippocampus, and amygdala) integrate cognitive, hedonic, and emotional stimuli in a non-homeostatic process (Berthoud 2011). Circulating energy balance signals, such as leptin and ghrelin, also target hedonic networks to modulate appetite. However, this system may override homeostatic control and cause energy imbalance (Berthoud 2011). In fact, striking similarities between food reward and drug addiction mechanisms have been reported (DiLeone *et al*. 2012). Therefore, these complex interactions between the homeostatic and non-homeostatic systems culminate in coordinated appetite and energy balance regulation through the modulation of endocrine, autonomic, and behavioral outputs (Fig. 2). The precise integrative mechanisms of these different levels of regulation and the generation of specific physiological outputs are among the main unsolved enigmas of the central regulation of energy balance.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review reported.

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