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.

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
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 \( \alpha \)-melanocyte-stimulating hormone (\( \alpha \)-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 \( \gamma \)-aminobutyric acid (GABA; Broberger et al. 1998, Cowley et al. 2001). The central administration of AGRP or its genetic over-expression 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 (Palmeter 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 \( \alpha \)-MSH, which exerts potent anorexigenic effects by binding to MC3R and MC4R (Mercer et al. 2013). The levels of Pomp transcripts and \( \alpha \)-MSH are increased under feeding conditions and decreased under fasting conditions (Schwartz et al. 1997). The i.c.v. administration of \( \alpha \)-MSH or its delivery into the PVN suppresses food intake and...
reduces body weight (Poggioli et al. 1986, Wirth et al. 2001). Genetic manipulation of the Pomp 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 Pomp, melanocortin peptides, or POMC neurons develop obesity (Yaswen et al. 1999, Gropp et al. 2005, Xu et al. 2005a, 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 (Pomch) 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
Neuronal circuits and energy balance

M Schneebberger and others

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 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 relaying 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 (Kolczynski 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 2009b). 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 2009a, 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 (Kirkhgsessner & 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...
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 therapeutic 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 Pmc 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 Pmc 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 (PPT1B) (Robertson et al. 2008). Consistent with this, deletion of either Socs3 or Ppt1b (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 p110α subunit in ARC neurons may play a more prominent role than p110α. PI3K generates phosphatidylinositol-3,4,5-triphosphate (PIP3) 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 PIP3. The loss of Pten in POMC neurons results in increased PIP3 signaling and diet-sensitive obesity via KATP 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. 2005b). Negative regulators of the LEPR, such as SOCS3 and PPT1B, also directly inhibit the IR and its signaling cascade acting on IRS1. The activation of the
<table>
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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 serine491 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,

<table>
<thead>
<tr>
<th>Genetic manipulation</th>
<th>Neuronal cell type</th>
<th>Energy expenditure</th>
<th>Diet</th>
<th>Other features</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>p85(Pik3r1) deletion</td>
<td>POMC</td>
<td>Chow</td>
<td>ND</td>
<td>Gender dimorphism on HFD administration</td>
<td>Hill et al. (2009)</td>
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<tr>
<td>p110alpha(Pik3ca) deletion</td>
<td>POMC</td>
<td>Chow</td>
<td>ND</td>
<td>Sensitive to HFD</td>
<td>Hill et al. (2009)</td>
</tr>
<tr>
<td>p110beta(Pik3cb) deletion</td>
<td>POMC</td>
<td>Chow</td>
<td>ND</td>
<td>Blunted insulin-induced depolarization</td>
<td>Hill et al. (2009)</td>
</tr>
<tr>
<td>p110gamma deletion</td>
<td>AgRP</td>
<td>Chow</td>
<td>ND</td>
<td>Blunted insulin-induced depolarization</td>
<td>Hill et al. (2009)</td>
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<tr>
<td>p110gamma deletion</td>
<td>POMC</td>
<td>Chow</td>
<td>ND</td>
<td>Neurons insensitive to glucose changes</td>
<td>Al-Qassab et al. (2009)</td>
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<tr>
<td>p110gamma deletion</td>
<td>AgRP</td>
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<td>Neurons insensitive to glucose changes</td>
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<td>ND, not determined.</td>
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Table 1 Continued
Circulating PYY levels are proportional to calorie intake and are reduced under fasting conditions (Adrian et al. 1985). Two endogenous forms, PYY₁₋₃₆ and PYY₃₋₃₆, 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 PYY₃₋₃₆, 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 z-MSH and reduced NPY release (Batterham et al. 2002). The effects of PYY₃₋₃₆ 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 Ppy 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
The 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, 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 melanocortinergic 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...
is called into question by the fact that the RIPcre transgene is also expressed in other regions of the brain and pancreatic b-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...
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 Ca²⁺ 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).
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. 2009b), 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 (Hrvath 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. 2009a). 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
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-kB 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 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 over-nutrition 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
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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