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Hypothalamic and brainstem neurocircuitries controlling homeostatic energy balance

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Abstract

Alterations in adequate energy balance maintenance results in serious metabolic disturbances such as obesity. In mammals, this complex process is orchestrated by multiple and distributed neuronal circuits. Hypothalamic and brainstem neurocircuitries are critically implicated in the sensing of circulating and local factors informing about the energy status of the organism. The integration of these signals culminates in the generation of specific and coordinated physiological responses aimed to regulate 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, electrophysioly and optogenetic techniques that have greatly contributed to improve our understanding of this central process.

1. Introduction

Appetite and body weight regulation are intricate processes 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 this system 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 this system in matching energy demand with 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 implicated in homeostatic regulation of energy balance.

2. The homeostatic system: hypothalamus and brainstem.

2.1. The hypothalamus: neuronal anatomy, nuclei and neuropeptides.

Seminal lesioning studies conducted in rodents during the 1940's and 50's highlighted the importance of the hypothalamus in body weight regulation. Since then, extensive experimental evidences 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 central nervous system (CNS) involved in energy homeostasis control. It is located below the VMN, on both sites of the third ventricle, and immediately adjacent to the median eminence (ME). This area has a semi-permeable blood brain barrier (BBB) (Broadwell and Brightman 1976), and thus it is strategically positioned to sense hormonal and nutrient fluctuations from 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 anorexigenic neuropeptides cocaine- and amphetamine regulated transcript (CART) and α-melanocyte stimulating hormone (α-MSH; a product of proopiomelanocortin (POMC) processing). These two populations of neurons (hereafter referred as AgRP and POMC, respectively), together with downstream target neurons expressing the melanocortin receptor 4 (MC4R) and 3 (MC3R), constitute the central melanocortin system. This neuronal circuit is crucial to sense and integrate a number of peripheral signals allowing for a precise control of food intake and energy expenditure (see section 4.1).

NPY is widely expressed throughout the CNS, but in the hypothalamus is most densely localized in the ARC (Gehlert, et al. 1987). ARC NPY expression and release respond to changes in energy status, being reduced in feeding conditions and increased with fasting (Beck, et al. 1990; Kalra, et al. 1991). Pharmacological increase of NPY tone results in hyperphagia and reduced thermogenesis of brown adipose tissue (BAT), associated with diminished activity of the thyroid axis (Clark, et al. 1984; Egawa, et al. 1991; Stanley, et al. 1986). Although NPY acts at 5 different receptors (Y1, Y2, Y3, Y4 and Y6 in mice), genetic and pharmacological studies suggest that postsynaptic Y1 and Y5 receptors mediate NPY effects on positive energy balance (Nguyen, et al. 2012; Sohn, et al. 2013).

AgRP is also an orexigenic neuropeptide, which is exclusively expressed in the ARC where colocalizes with NPY and the neurotransmitter γ-aminobutyric acid (GABA) (Broberger, et al. 1998; Cowley, et al. 2001). 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 to the PVN, DMN and LHA. Despite the well documented effects of NPY and AgRP as positive modulators of energy balance, genetic studies have provided conflicting results. For example, *AgRP* and *Npy* knock-out (KO) mice failed to exhibit alterations in body weight or feeding behavior (Corander, et al. 2011; Palmiter, et al. 1998; Qian, et al. 2002). However, ablation of AgRP neurons in adult leads to uncontrolled anorexia but is well tolerated in neonates, suggesting 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 is particularly abundant in the hypothalamus and in the ARC colocalizes (>95%) with POMC (Elias, et al. 1998). Its expression is enhanced by feeding and reduced under fasting

conditions (Kristensen, et al. 1998), and it has been shown that intracerebroventricular (icv) infusion of CART inhibits food intake while antibodies against CART reverse these effects (Kristensen et al. 1998). Furthermore, CART also stimulates BAT thermogenesis (Kotz, et al. 2000). However, CART deficient mice show no alterations in food intake or body weight when fed with a standard diet, but develop obesity after high-fat diet (HFD) administration (Asnicar, et al. 2001). Interestingly, and contrary to the prevailing anorexigenic view, other studies have evidenced that under certain experimental conditions CART may stimulate food intake (Abbott, et al. 2003; Kong, et al. 2003). Collectively, the effects of CART on feeding behavior are inconclusive and suggest anatomically divergent roles for this neuropeptide.

POMC is a prohormone precursor that in the hypothalamus is cleaved into several bioactive peptides, including α-MSH which exerts potent anorexigenic effects through binding to MC3R and MC4R (Mercer, et al. 2013). POMC transcript and α-MSH levels are increased by feeding and decreased by fasting (Schwartz, et al. 1997). Icv administration of α-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 *Pomc* gene leading to α-MSH over expression showed anti-obesity effects in genetic and diet-induced obesity (DIO) models (Lee, et al. 2007; Mizuno, et al. 2003; Savontaus, et al. 2004). A key role for POMC in whole-body energy homeostasis is evident, as mice lacking POMC, melanocortin peptides or POMC neurons develop obesity (Gropp et al. 2005; Smart, et al. 2006; Xu, et al. 2005a; Yaswen, et al. 1999). Furthermore, mutations in the POMC gene have been 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 role is 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 MC3/4R. It receives innervation mainly from ARC AgRP and POMC neurons, but also from extrahypothalamic regions such as the nucleus of the tractus solitarius (NTS). The PVN is an important integration site implicated 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 or α-MSH amongst others (Kim, et al. 2000; Stanley et al. 1986). 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; Fekete, et al. 2004). Another relevant subset of neurons express corticotrophin releasing hormone (CRH), which are directly implicated in energy balance control through AgRP innervation or indirectly through regulation of adrenal glucocorticoids controlling POMC expression (Richard and Baraboi 2004).

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

Dorsomedial nucleus: the DMN is implicated in a range of physiological processes, including feeding, thermoregulation, stress and circadian rhythms. It receives projections from most hypothalamic nuclei, specially the ARC, and sends innervations to the PVN and LHA. A number of neuropeptides (such as NPY and CRH) as well as receptors for peptides implicated in appetite and energy balance control are expressed within the DMN. Increased NPY expression in the DMN has been reported in several rodent models of obesity

(Bi, et al. 2001; Guan, et al. 1998), and may play a significant role in thermogenesis regulation and the development of DIO (Chao, et al. 2011).

Ventromedial nucleus: ARC AgRP and POMC neurons project to the VMN. In turn, VMN neurons project to 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 (SF-1) which has been directly implicated in the development of the VMN (Davis, et al. 2004; Parker, et al. 2002). SF-1 expressing neurons play significant roles in energy balance control, as demonstrated by the metabolic phenotypes of conditional KO mice (Bingham, et al. 2008; Kim, et al. 2011; Zhang, et al. 2008). Another abundantly expressed protein in the VMN is the brain derived neurotrophic factor (BDNF). Lack of BDNF or its receptor (TRKB) leads to hyperphagia and obesity in humans and mice (Lyons, et al. 1999; Yeo, et al. 2004). In contrast, central or peripheral BDNF administration produces body weight loss and reduction in food intake through MC4R signaling (Xu, et al. 2003). The VMN also plays a key role in thermogenesis regulation (Kim et al. 2011; Lopez, et al. 2010; Martinez de Morentin, et al. 2012; Whittle, et al. 2012).

2.2. The brainstem

Brainstem neurons make key contributions to the energy balance control by processing energy-status information at four different levels: 1) by sensing circulating metabolites and hormones released by peripheral organs; 2) by receiving vagal inputs from the gastrointestinal (GI) tract; 3) by receiving neuronal inputs from midbrain and forebrain nuclei that also detect and integrate energy-related signals; 4) by projecting to local brainstem circuits and other brain regions to provide information that will be integrated by those neurons to control energy balance. Within the brainstem, the dorsal vagal complex (DVC) is a key module for integration of energy-related cues by

relying peripheral signals through vagal afferents and projecting to the hypothalamus and other relevant areas. The DVC comprises the dorsal motor nucleus of the vagus (DMV), 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 or POMC amongst 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 (GLP-1) or 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 to 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 (Gil, et al. 2011; Krolczyk, et al. 2001). Vagal signaling also plays important functions in regulating meal size and duration (Schwartz, et al. 1999).

The NTS receives inputs from descending projections from the hypothalamus. In particular, ARC POMC neurons project to the NTS where high expression levels of MC4R have been reported (Kishi, et al. 2003). In addition to α-MSH release 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 and 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 feeding and an increase in energy expenditure whereas MC4R antagonism drives the opposite effect (Skibicka and Grill 2009b; Williams, et al. 2000). MC4R's in the NTS seem to mediate the satiation effects of CCK (Fan, et al. 2004), but also the anorexigenic effects of hypothalamic and brainstem leptin signaling (Skibicka and 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 orexin A delivery in the hindbrain increases food intake (Parise, et al. 2011). The orexigenic nature of the LHA, and the anatomical connection with the NTS, suggest that this system may serve as a mechanism to limit the satiety signals from the GI tract.

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

3. Hormonal signals implicated in energy homeostasis control

3.1. 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 isoforms, being the long form (LepRb) essential for leptin effects. Lack of leptin or

LepRb in both rodents and humans causes a phenotype characterized by hyperphagia, reduced energy expenditure and severe obesity (Chen, et al. 1996; Clement, et al. 1998; Halaas, et al. 1995; Montague, et al. 1997). 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 implicated in energy balance control (Elmquist et al. 1998). In the ARC, POMC and AgRP neurons are direct targets of leptin (Cheung, et al. 1997; Cowley et al. 2001; Elias, et al. 1999). Ablation of LepRb in POMC, AgRP or both populations of neurons cause increased body weight, emphasizing the importance of leptin signaling (Table 1). However, the magnitude of these changes are smaller than those observed in mice globally lacking LepR, suggesing the existence of additional subsets of neurons mediating leptin effects on food intake and body weight. Leptin binds to LepRb and activates Janus kinase 2 (JAK-2) which, in turn, phosphorylates several tyrosine residues on the intracellular domain of the LepRb. This results in the activation, dimerization and nuclear translocation of signal transducer and activator of transcription 3 (STAT-3) (Robertson, et al. 2008). In the nucleus, STAT-3 enhances *Pomc* and inhibits *Agrp* gene expression (Kitamura, et al. 2006; Munzberg, et al. 2003). Accordingly, STAT-3 deficiency in POMC neurons results in overweight and *Pomc* gene transcriptional defects in females (Table 1). This signaling cascade is negatively regulated by suppressor of cytokine signaling 3 (SOCS-3), the expression of which is also regulated by STAT-3, and protein tyrosine phosphatase 1B (PTP-1B) (Robertson et al. 2008). Consistent with this, deletion of either SOCS-3 or PTP-1B in POMC neurons led 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 (PDK-1) and AKT (also known as protein kinase B) which consecutively phosphorylates the transcription factor forkhead box protein O1 (FOXO-1). Upon phosphorylation, FOXO-1 is excluded from the nucleus allowing STAT-3 to bind *Pomc* and *Agrp* promoters, thereby stimulating and inhibiting respectively the expression of these neuropeptides (Kitamura et al. 2006). These findings are in line with genetic manipulations in vivo (Table 1). PI3K signaling is counterbalanced by phosphatase and tensin homolog (PTEN), which specifically dephosphorylates PIP_3. Loss of PTEN in POMC neurons resulted in increased PIP_3 signaling and diet-sensitive obesity via KATP channel modulation, suggesting a role for PI3K pathway in the regulation of the activity of this channel (Table 1). Overall, leptin stimulates transcription of *Pomc*, depolarizes POMC neurons and also increases α-MSH processing and secretion (Cowley et al. 2001; Guo, et al. 2004; Munzberg et al. 2003) while attenuates the expression and release of orexigenic NPY and AgRP neuropeptides (Mizuno and Mobbs 1999; Stephens, et al. 1995).

Insulin, produced by pancreatic β-cells, has been traditionally 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). Icv or intrahypothalamic administration of insulin to primates and rodents reduces food intake (Air, et al. 2002; McGowan, et al. 1993; Woods, et al. 1979). Insulin receptor (IR), as well as its downstream signaling machinery, is expressed in hypothalamic areas implicated in feeding control (Corp, et al. 1986; Havrankova, et al. 1978) and colocalize with AgRP and POMC neurons (Benoit, et al. 2002). Surprisingly, loss of IR in either POMC or AgRP neurons does not cause alterations in energy balance (Table 1) although hepatic glucose production defects were observed in mice lacking IR in AgRP neurons (Konner, et al. 2007a). 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 control glucose metabolism and energy expenditure respectively (Table 1). Insulin binding to IR leads to receptor autophosphorylation and the consequent recruitment of IRS proteins, which converges with leptin pathway at PI3K level (Xu, et al. 2005b). Negative regulators of the leptin receptor, such as SOCS-3 and PTP-1B, also directly inhibit the IR and its signaling cascade acting on IRS-1. The activation of the IR signaling pathway results in reduced expression of NPY and increased POMC levels in the ARC thus stimulating an anorexigenic effect (Benoit et al. 2002; Schwartz, et al. 1992; Sipols, et al. 1995).

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

3.2. Gastrointestinal hormones

Ghrelin is a 28 aminoacid 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 is increased by fasting and reduced after refeeding (Tschop, et al. 2000). Central and peripheral administration of ghrelin in rodents robustly promoted feeding, adiposity and body weight gain (Nakazato, et al. 2001; Tschop et al. 2000). 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 to mediate

ghrelin's orexigenic effects (Chen, et al. 2004). Ghrelin is able to stimulate *Npy* and *AgRP* transcription, but also increases the number of stimulatory synapses on AGRP neurons while increases the number of inhibitory synapses on POMC neurons (Cowley, et al. 2003; Kamegai, et al. 2001; Nakazato et al. 2001). However, neuronal activation and positive energy balance has been also reported after ghrelin administration in the PVN, LHA, hindbrain and 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 (Adrian, et al. 1985; Tatemoto and Mutt 1980). Circulating PYY levels are proportional to the 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 inhibition of ARC Y2 receptors, as demonstrated by pharmacological (Abbott, et al. 2005; Scott, et al. 2005) and genetic studies (Batterham et al. 2002), that eventually lead 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 peripheral delivery of this peptide increased neuronal activity in NTS and AP neurons and stimulated vagal afferent firing (Blevins, et al. 2008; Koda, et al. 2005). Consistent with a role for PYY in appetite and body weight regulation, transgenic mice globally lacking or overexpressing PYY exhibited opposite alterations in energy balance control (Batterham, et al. 2006; Boey, et al. 2008).

GLP-1, the cleavage product of proglucagon in the intestine and brain, is mainly secreted from intestinal L-cells. Similar to PYY, GLP-1 circulating levels are high following a meal and are low in fasted conditions. This hormone exerts a strong incretin effect, via GLP-1 receptors (GLP-1R) expressed in pancreatic islets, enhancing insulin secretion after carbohydrate ingestion (Kreymann, et al. 1987). GLP-1R is also expressed in key CNS areas

implicated in energy balance control, such as the hypothalamus and brainstem (Merchenthaler, et al. 1999). A number of studies have shown that central or site-specific administration of GLP-1 or GLP-1 analogues inhibits food intake in rodents (Hayes, et al. 2008; McMahon and Wellman 1998; Tang-Christensen, et al. 1996; Turton, et al. 1996). Interestingly, neurons containing proglucagon gene are present in the NTS suggesting the existence of a local circuit implicated in appetite control (Merchenthaler et al. 1999). In fact, recent studies provide evidence for a dual (peripheral and central) role of GLP-1 in appetite suppression 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 and Smith 1977; Gibbs, et al. 1973; Kissileff, et al. 1981). CCK 1 and 2 receptors are expressed in brainstem and hypothalamus, but CCK anorectic effects are critically mediated by vagal sensory neurons that project to 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 feeding (Fan et al. 2004). It has been also reported that ghrelin attenuates and leptin synergistically potentiates CCK effects on appetite (Barrachina, et al. 1997; Lee, et al. 2011).

4. 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 implicated in the regulation of whole-body energy homeostasis. For example, in a post-absorptive situation circulating cues of energetic surfeit (leptin, insulin, GLP-1, PYY, 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 in order 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.

4.1. ARC neuronal circuits: POMC, AgRP and RIPCre neurons

Melanocortin peptides and NPY are two basic components of a critical hypothalamic circuit implicated in the convergence and integration of nutritional and hormonal cues aimed to regulate organismal energy balance. ARC POMC and AgRP neurons are located in close proximity to each other and project in parallel to similar brain areas expressing MCRs. Both POMC and AgRP neurons are able to sense a number of peripheral (leptin, insulin, ghrelin) and central (NPY, GABA, serotonin, melanocortins) 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 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 and Monck 2001; Nijenhuis, et al. 2001; Tolle and Low 2008). Indeed, substantial experimental evidence indicates that agonism of MCRs attenuates appetite and enhances energy expenditure, whereas their antagonism have essentially the opposite effects (Fan, et al. 1997; Harrold, et al. 1999; Hwa, et al. 2001). This is consistent with data showing that loss or mutations in MC3R and MC4R genes cause obesity both in rodents and humans (Butler, et al. 2000; Farooqi 2008; Huszar, et al. 1997). In addition to inhibit 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 and activating POMC neurons (Figure 1A). Leptin enhances *Pomc* gene expression and processing into α-MSH (Mizuno, et al. 1998; Schwartz et al. 1997; Thornton, et al. 1997). Electrophysiology studies have demonstrated that local-applied leptin is able to depolarize (excite) POMC neurons (Al-

Qassab, et al. 2009; Claret et al. 2007; Claret, et al. 2011; Cowley et al. 2001; Hill, et al. 2008; Qiu, et al. 2010) likely through TRPC channels (Qiu et al. 2010). In contrast, leptin inhibits *Npy* and *AgRP* gene transcription in the hypothalamus (Mizuno and Mobbs 1999; Schwartz, et al. 1996; Stephens et al. 1995). Electrophysiological recordings have shown that leptin decreases the GABAergic-mediated tone exerted by AgRP neurons onto neighboring POMC neurons, resulting in a disinhibition of POMC neuron activity (Cowley et al. 2001). The ability of leptin to directly hyperpolarize (inhibit) AgRP neurons is controversial (Al-Qassab et al. 2009; Claret et al. 2007; Cowley et al. 2001), but studies in rat 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 onto postsynaptic POMC neurons thus further contributing to maintain the anorexigenic actions mediated by this hormone (Figure 1A) (Vong, et al. 2011).

On the other hand, under conditions of negative energy balance, circulating ghrelin levels are increased. Ghrelin actions 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 increases the number of GABAergic inhibitory synapses on POMC neurons (Figure 1B) (Atasoy, et al. 2012; Cowley et al. 2003; van den Pol, et al. 2009; Yang, et al. 2011). The importance of this GABAergic stimuli on energy balance control has been substantially demonstrated (Horvath, et al. 1997; Wu, et al. 2009; Wu, et al. 2012; Wu and Palmiter 2011) and conditional deletion of the vesicular GABA transporter in AgRP neurons blunts the inhibitory tone onto postsynaptic POMC neurons leading to enhanced melanocortigenic output and lean phenotype (Tong, et al. 2008). Moreover, AgRP and NPY directly hyperpolarize POMC neurons and decrease α-MSH production and release, further inhibiting the activity of this population of neurons (Cyr, et al. 2013; Roseberry, et al. 2004; Smith, et al. 2007). Thus, AgRP neurons are able to negatively modulate the anorexigenic

effects of POMC neurons by direct (GABAergic synapsis) and indirect (MCR antagonism) mechanisms (Figure 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 from Horvath lab, 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 been recently reviewed in detail (Dietrich and Horvath 2013; Zeltser, et al. 2012).

A novel subpopulation of ARC neurons involved in energy balance control (defined by virtue of Cre-mediated expression of rat insulin II promoter-Cre transgene and called RIPCre neurons) has been recently 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 suggest that RIPcre neurons may be targets of POMC and/or AgRP neurons. Indeed, bath application of a melanocortin agonist caused a direct long-lasting depolarization and increased firing in ARC RIPcre neurons (Choudhury et al. 2005). Interestingly, insulin also depolarized these neurons while leptin did not cause any electrophysiological effect (Choudhury et al. 2005).

Although a number of mouse genetic studies suggest that ARC RIPCre neurons regulate systemic energy balance (Choudhury et al. 2005; Cui, et al. 2004), this interpretation is curtailed by the fact that the RIPcre transgene is also expressed in other brain regions and pancreatic β-cells. However, recent data showed that acute and selective ablation of ARC RIPcre neurons leads to hypophagia, reduced food intake and adiposity through compensatory increase 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 have shown that synaptic release of GABA, but not glutamate, from this subset of neurons increase BAT thermogenic function

without affecting food intake (Kong, et al. 2012). The effects of leptin on RIPcre neurons is 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, leptin's ability to increase energy expenditure is impaired in mice lacking vesicular GABA transporter in RIPcre neurons indicating a functional effect of this hormone on this neurons (Kong et al. 2012).

Taken together, current evidence suggests that a local ARC circuit constituted by "first-order" POMC, AgRP and RIPcre neurons plays a key role in integrating humoral signals reporting on energy conditions. This is achieved by a sophisticated and multilevel organizational structure that allows accurate regulation of orexigenic and anorexigenic outputs through direct and indirect mechanisms.

4.2. 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. This receptor is localized in many nuclei implicated in the regulation of energy balance where POMC and AgRP neurons send axon projections. MC4Rs are Gs-protein-coupled receptors that stimulate adenylyl cyclase thereby increasing intracellular cAMP (Florijn, et al. 1993). A series of elegant studies using a cell-specific MC4R reexpression 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) (Figure 1A). Furthermore, and contrary to the prevailing view, a recent report shows that POMC neurons also express MC4Rs which 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, exerted by α-MSH released from the same cell and/or neighbor 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, insulin release) functions (Figure 1A).

NPY receptors are Gi/o-protein-coupled receptors that reduce cAMP production, leading to activation of G-protein-gated inward rectifying K^+(GIRK) channels and inhibition of voltage-dependent Ca^{2+} channels (VDCC) (Sohn et al. 2013). The precise roles of NPY receptors and their contribution in mediating the orexigenic effects of NPY have been difficult to delineate due to the paradoxical phenotypes of receptor KO mouse models. This is likely the consequence of receptor redundancies and compensatory mechanisms exhibited by germ-line deletion strategies. Despite these limitations, pharmacological and genetic studies suggest that NPY orexigenic actions are mediated by postsynaptic Y1 and Y5 within the PVN (Nguyen et al. 2012; Sohn et al. 2013) (Figure 1B). Of note, 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 (PC2)-mediated pro-TRH processing in the PVN through Y1/Y5 receptors (Cyr et al. 2013). Taken together, abundant evidence suggests 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 energy balance regulation.

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

Most of the experimental evidences 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 have provided a way to exert temporally and spatially precise control over the activity of defined circuit elements. This permits to establish causal connections between circuit activity and behavioral responses (Sternson 2013).

Using an elegant combination of optogenetics and mouse genetics approaches, Aponte and collaborators have confirmed that selective activation of AgRP neurons are sufficient to evoke voracious feeding in mice, without previous training and independent of melanocortin signaling (Aponte, et al. 2011). The level of neuronal activation was correlated with the magnitude, dynamics and duration of the induced behavioral response. Furthermore, continuous photostimulation was required to maintain evoked feeding suggesting that 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 resulted in reduced food intake and body weight gain that required downstream MC4R activity (Aponte et al. 2011).

The behavioral effects on food intake caused by AgRP or POMC neuron activation were 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 behavior 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 was necessary to reduce appetite. Interestingly, acute stimulation of NTS POMC neurons generated an immediate suppression of food intake (Zhan et al. 2013).

In a subsequent study, the Sternson group performed a series of experiments to find out which brain regions and cell-types mediate evoked feeding from 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). Of note, the authors found that ARC AgRP neurons induce evoke feeding through inhibitory input onto oxytocin neurons in the PVN while ARC POMC neurons are implicated in long-term control of appetite and energy balance (Atasoy et al. 2012).

Collectively, these results emphasize a previously unrecognized importance for temporal and spatial activation of POMC and AgRP neurons.

Thus, ARC AgRP and NTS POMC neurons would be implicated in the regulation of acute feeding, while ARC POMC neurons may be involved in long-term responses. This demonstrate 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 in order to precisely define the importance of these factors and to reconcile these observations with previous evidences (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.

5. New players in energy balance control

5.1. 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-shape cells that are involved in a number of functions, such as metabolic support to neurons, transmitter uptake and release as well as synaptic remodeling (Sofroniew and Vinters 2010). Astrocytes express LepR (Cheunsuang and 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 (Fuente-Martin, et al. 2012; Garcia-Caceres, et al. 2011). This may cause changes in 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 neurons perikarya (Horvath, et al. 2010). It has been also reported that DIO mice exhibit increased expression of

functional astrocytic LepR in the hypothalamic region, an effect that may play a role in leptin resistance development (Hsuchou, et al. 2009a). Indeed, loss of astrocytic LepR under HFD conditions provides a partial protection to develop disturbances in neuronal leptin signaling (Jayaram, et al. 2013).

Obesity and lipid overload induces 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 play 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 been also reported in rodents and primates with 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 close proximity with neurons in the ARC and VMN (Bolborea and Dale 2013). Although it is unknown whether tanycytes are able to modulate the activity of hypothalamic neurons, several lines of evidence suggest that this particular cell type may be implicated 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 and Dale 2013) and regulate the permeable properties of the fenestrated capillaries of the ME which may constitute a way to modulate 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, manipulation of hypothalamic neurogenesis in adult mice 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). In contrast, genetic IKKβ/NF-κB activation in Sox2 positive hypothalamic cells lead to overeating and weight gain (Li et al. 2012). It is important to note that these strategies did not exclusively target tanycytes, so these metabolic effects can not 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 and Dale 2013).

5.2. Epigenetic mechanisms

The interplay between genetic and environmental factors (nutrition, maternal health, chemicals, lifestyle, etc.) during prenatal or perinatal periods and their influence in the development of energy balance and metabolic alterations into adulthood has recently received substantial interest. In both humans and animal models, prenatal or perinatal nutrional manipulations lead to chronic metabolic disturbances in terms of feeding behavior, energy expenditure, leptin sensitivity or 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, post-translational histone modifications and non-coding RNAs. Neonatal overfeeding in rats, which results in overweight and metabolic syndrome, is associated with POMC gene promoter hypermethylation (Plagemann, et al. 2009). The extent of this DNA methylation is negatively correlated with POMC expression in relation to leptin and insulin levels, suggesting functionality of

acquired epigenomic alterations (Plagemann et al. 2009). In the same overnutrition model, Plagemann and collaborators also found increased methylation of the IR promoter in the hypothalamus (Plagemann, et al. 2010). Similarly, epigenetic remodeling of hypothalamic genes induced by mild maternal undernutrition (Begum, et al. 2012; Stevens, et al. 2010) or stress (Paternain, et al. 2012) have also been 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, these evidences support 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 implicated in energy balance control. Nevertheless, further research is warranted in order to address the significance of these epigenetic events.

MicroRNAs (miRNAs), a class of small, non-coding RNAs that regulate gene expression at post-transcriptional level, have been recently suggested to be involved in the hypothalamic control of energy balance. It has been demonstrated that the expression of Dicer, an essential endoribonuclease for miRNA maturation, is regulated by nutrient availability and excess in the hypothalamus (Schneeberger, et al. 2012). Furthermore, we have also shown that deletion of Dicer 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 (Schneeberger, et al. 2012, Greenman, 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 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 upon whole-body energy balance regulation still remains unknown.

6. Concluding remarks: neuronal circuitry integration and physiological responses

As 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 to fulfill 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 (Figure 2). The precise integrative mechanisms of these different levels of regulation and the generation of specific physiological outputs is one of the main unsolved enigmas of the central regulation of energy balance.

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Figure Legends

Figure 1. Schematic representation of the main neuronal circuits engaged by leptin and ghrelin. (**A**) Leptin is released in proportion to fat stores and in the ARC stimulates the activity of anorexigenic POMC neurons while inhibits neighbor AgRP neurons. This results in increased α-MSH release and the activation of downstream second-order neurons expressing MC4R in hypothalamic and extrahypothalamic regions. POMC neurons also express MC4R, suggesting the existence of an autoregulatory mechanism exerted 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 enhance 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. Green arrows: activation effect. WAT: white adipose tissue.

Figure 2. Schematic integration of the different levels of food intake and energy balance regulation. Food intake and energy balance is 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 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 likely through non-homeostatic and higher brain structures. LHA: lateral hypothalamic area; VTA: ventral tegmental area; NAc: nucleus accumbens.

Table 1. Summary of relevant genetic mouse models used in the analysis of leptin and insulin signaling pathways in POMC and AgRP neurons. N/D: not determined.

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	=	=	=	N/D	Chow and HFD	-	(Konner, et al. 2007b)
IR deletion	AgRP	=	=	=	N/D	Chow and HFD	Enhaced hepatic glucose production	(Konner et al. 2007b)
IR reexpression in L1 mice	POMC	-	=	+	+	Chow	Insulin resistance	(Lin, et al. 2010)
IR reexpression in L1mice	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)
IRS-2 deletion	POMC	=	=	=	=	Chow	Normal insulin and leptin levels	(Choudhury et al. 2005)

PTP1-B deletion	POMC	-	-	=	+	HFD	Improved leptin sensitivity	(Banno, et al. 2010)
STAT-3 deletion	POMC	+	+	+	N/D	Chow	Normal phenotype in male mice	(Xu, et al. 2007)
STAT-3 deletion	AgRP	+	+	+	N/D	Chow	Hyporesponsive to leptin	(Gong, et al. 2008)
STAT-3 constitutive active form	POMC	+	+	+	N/D	Chow	No additional effect on HFD	(Ernst, et al. 2009)
STAT-3 constitutive active form	AgRP	-	-	=	+	Chow and HFD	Increased locomotor activity	(Mesaros, et al. 2008)
PDK-1 deletion	POMC	+	+	+	=	Chow	Decreased POMC gene expression	(Iskandar, et al. 2010)
PDK-1 deletion	AgRP	-	-	-	=	Chow	Rescued by dominant negative Foxo1	(Cao, et al. 2011)
PDK-1 deletion	POMC	transient +	transient +	transient +	N/D	Chow and HFD	Rescued by dominant negative Foxo1	(Belgardt, et al. 2008)
FOXO-1 deletion	POMC	-	-	-	=	Chow	Increased Cpe expression and a-MSH levels	(Plum, et al. 2009)

FOXO-1 constitutive active form	POMC	+ (females)	+ (females)	+ (females)	=	Chow	Decreased POMC gene expression	(Iskandar et al. 2010)
FOXO-1 deletion	AgRP	=	-	-	=	Chow	Resistant to HFD	(Ren, et al. 2012)
SOCS-3 deletion	POMC	-	N/D	=	+	HFD	No body weight phenotype on chow diet	(Kievit, et al. 2006)
SOCS-3 overexpression	POMC	+	+	=	-	Chow	Leptin resistance	(Reed, et al. 2010)
SOCS-3 overexpression	AgRP	=	=	+	+	Chow	Altered glucose metabolism	(Olofsson, et al. 2013)
PTEN deletion	POMC	+	+	+	=	Chow	Gender dimorphism on HFD	(Plum et al. 2009)
p85 deletion	POMC	=	N/D	N/D	N/D	Chow	Gender dimorphism on HFD	(Hill, et al. 2009)
p110 α deletion	POMC	+	+	=	- (females)	Chow	Sensitive to HFD	(Hill et al. 2009)
p110 α deletion	POMC	=	=	=	=	Chow	Sensitive to HFD	(Al-Qassab et al. 2009)
p110 α deletion	AgRP	=	=	=	=	Chow and HFD	Blunted insulin-induced depolarization	(Al-Qassab et

								al. 2009)
p110B deletion	POMC	=	+	+	=	Chow	Sensitive to HFD	(Al-Qassab et al. 2009)
p110B deletion	AgRP	-	-	-	=	Chow and HFD	Blunted insulin-induced depolarization	(Al-Qassab et al. 2009)
AMPKα2 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)

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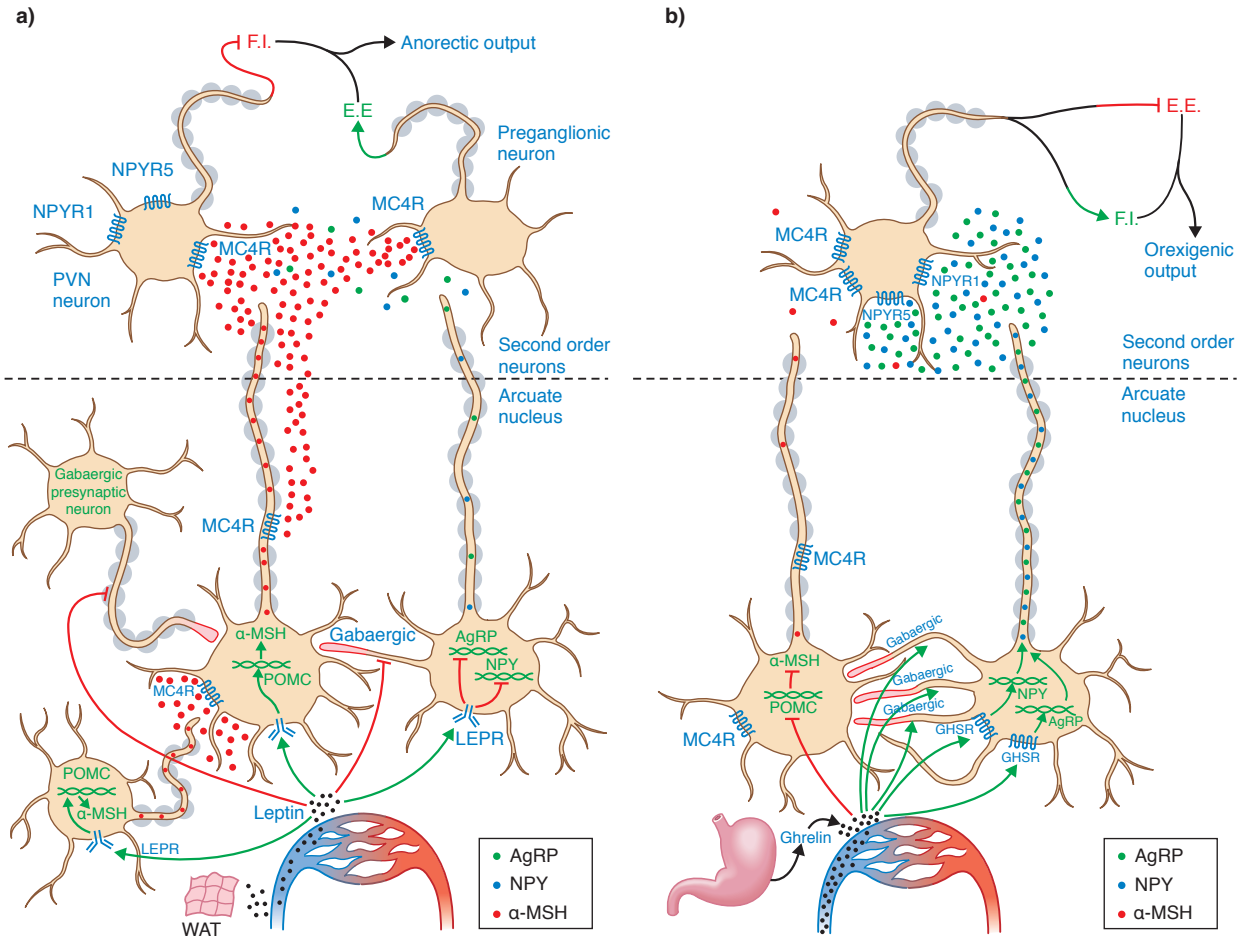
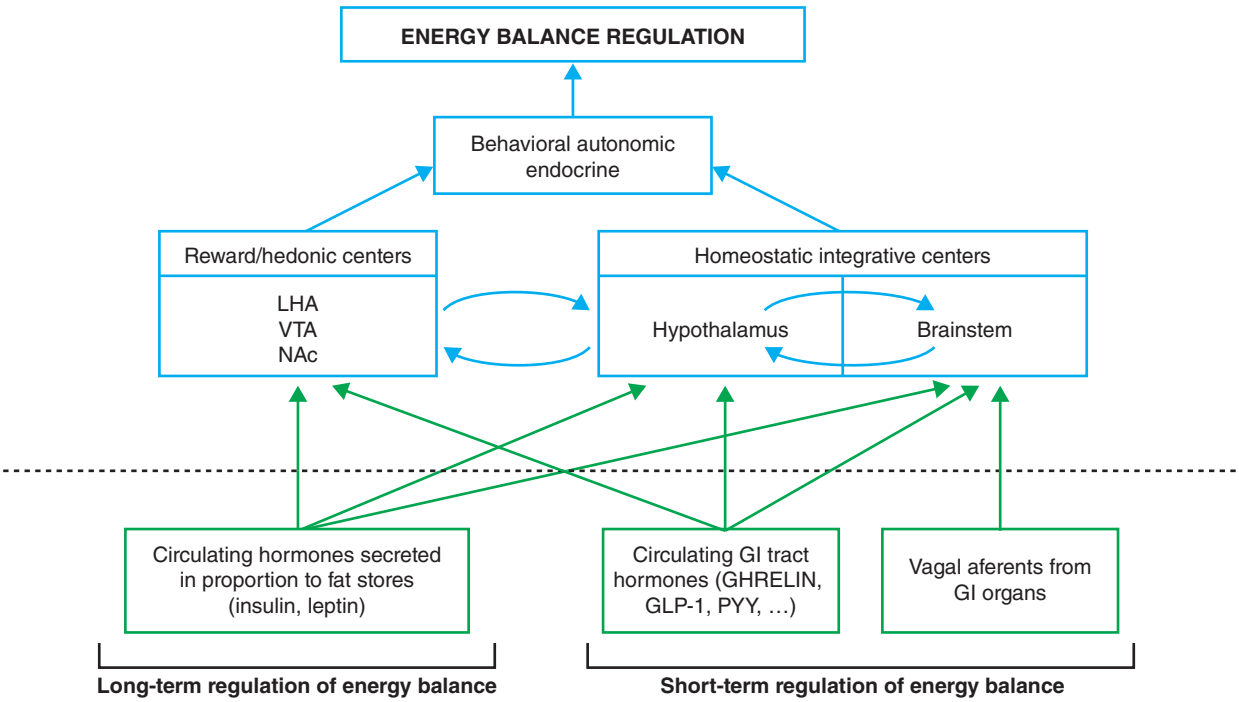

a)
F.I.
Anorectic output
E.E
Preganglionic neuron
NPYR5
NPYR1
MC4R
MC4R
PVN neuron
Second order neurons
Arcuate nucleus
Gabaergic presynaptic neuron
MC4R
α-MSH
Gabaergic
AgRP
NPY
MC4R
POMC
LEPR
POMC
α-MSH
Leptin
LEPR
WAT
AgRP
NPY
α-MSH
b)
E.E.
F.I.
Orexigenic output
MC4R
MC4R
NPYR1
NPYR5
Second order neurons
Arcuate nucleus
MC4R
α-MSH
Gabaergic
Gabaergic
Gabaergic
NPY
AgRP
POMC
MC4R
GHSR
GHSR
Ghrelin
AgRP
NPY
α-MSH


ENERGY BALANCE REGULATION
Behavioral autonomic endocrine
Reward/hedonic centers
LHA
VTA
NAc
Homeostatic integrative centers
Hypothalamus
Brainstem
Circulating hormones secreted in proportion to fat stores (insulin, leptin)
Circulating GI tract hormones (GHRELIN, GLP-1, PYY, …)
Vagal aferents from GI organs
Long-term regulation of energy balance
Short-term regulation of energy balance

Genetic Manipulation	Neuronal Cell type	BW	Adiposity	Food Intake	Energy Expenditure	Diet	Other features	References
LepR deletion	POMC	+	+	=	=	Chow	Altered neuropeptide expression	{Balthasar, 2004 #976}
LepR deletion	AgRP	+	+	=	=	Chow	Reduced locomotor activity	{van de Wall, 2008 #1399}
LepR deletion	POMC and AgRP	+	+	transient +	-	Chow	Increased respiratory exchange ratio	{van de Wall, 2008 #1399}
IR deletion	POMC	=	=	=	N/D	Chow and HFD	-	{Konner, 2007 #1128}
IR deletion	AgRP	=	=	=	N/D	Chow and HFD	Enhaced hepatic glucose production	{Konner, 2007 #1128}
IR reexpression in L1 mice	POMC	-	=	+	+	Chow	Insulin resistance	{Lin, 2010 #1129}
IR reexpression in L1mice	AgRP	-	=	=	+	Chow	Rescued hepatic glucose production	{Lin, 2010 #1129}
LepR and IR deletion	POMC	+	=	=	-	Chow	Insulin resistance and reduced fertility in females	{Hill, 2010 #280}
IRS-2 deletion	POMC	=	=	=	=	Chow	Normal insulin and leptin levels	{Choudhury, 2005 #859}
PTP1-B deletion	POMC	-	-	=	+	HFD	Improved leptin sensitivity	{Banno, 2010 #1401}
STAT-3 deletion	POMC	+	+	+	N/D	Chow	Normal phenotype in male mice	{Xu, 2007 #261}
STAT-3 deletion	AgRP	+	+	+	N/D	Chow	Hyporesponsive to leptin	{Gong, 2008 #1402}
STAT-3 constitutive active form	POMC	+	+	+	N/D	Chow	No additional effect on HFD	{Ernst, 2009 #1403}
STAT-3	AgRP	-	-	=	+	Chow and HFD	Increased locomotor activity	{Mesaros,

constitutive active form								2008 #1404}
PDK-1 deletion	POMC	+	+	+	=	Chow	Decreased POMC gene expression	{Iskandar, 2010 #1405}
PDK-1 deletion	AgRP	-	-	-	=	Chow	Rescued by dominant negative Foxo1	{Cao, 2011 #1406}
PDK-1 deletion	POMC	transient +	transient +	transient +	N/D	Chow and HFD	Rescued by dominant negative Foxo1	{Belgardt, 2008 #196}
FOXO-1 deletion	POMC	-	-	-	=	Chow	Increased Cpe expression and a-MSH levels	{Plum, 2009 #276}
FOXO-1 constitutive active form	POMC	+ (females)	+ (females)	+ (females)	=	Chow	Decreased POMC gene expression	{Iskandar, 2010 #1405}
FOXO-1 deletion	AgRP	=	-	-	=	Chow	Resistant to HFD	{Ren, 2012 #1408}
SOCS-3 deletion	POMC	-	N/D	=	+	HFD	No body weight phenotype on chow diet	{Kievit, 2006 #40}
SOCS-3 overexpression	POMC	+	+	=	-	Chow	Leptin resistance	{Reed, 2010 #1416}
SOCS-3 overexpression	AgRP	=	=	+	+	Chow	Altered glucose metabolism	{Olofsson, 2013 #1407}
PTEN deletion	POMC	+	+	+	=	Chow	Gender dimorphism on HFD	{Plum, 2009 #276}
p85 deletion	POMC	=	N/D	N/D	N/D	Chow	Gender dimorphism on HFD	{Hill, 2009 #281}
p110 α deletion	POMC	+	+	=	- (females)	Chow	Sensitive to HFD	{Hill, 2009 #281}
p110 α deletion	POMC	=	=	=	=	Chow	Sensitive to HFD	{Al-Qassab, 2009 #842}
p110 α deletion	AgRP	=	=	=	=	Chow and HFD	Blunted insulin-induced depolarization	{Al-Qassab,

								2009 #842}
p110B deletion	POMC	=	+	+	=	Chow	Sensitive to HFD	{Al-Qassab, 2009 #842}
p110B deletion	AgRP	-	-	-	=	Chow and HFD	Blunted insulin-induced depolarization	{Al-Qassab, 2009 #842}
AMPKα2 deletion	POMC	+	+	+ after fast	-	Chow and HFD	neurons insensitive to glucose changes	{Claret, 2007 #1050}
AMPKα2 deletion	AgRP	-	=	=	=	Chow	neurons insensitive to glucose changes	{Claret, 2007 #1050}