

# Thoughts for Food: Brain Mechanisms and Peripheral Energy Balance

## Review

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The past decade has witnessed dramatic advancements regarding the neuroendocrine control of food intake and energy homeostasis and the effects of peripheral metabolic signals on the brain. The development of molecular and genetic tools to visualize and selectively manipulate components of homeostatic systems, in combination with well-established neuro-anatomical, electrophysiological, behavioral, and pharmacological techniques, are beginning to provide a clearer picture of the intricate circuits and mechanisms of these complex processes. In this review, we attempt to provide some highlights of these advancements and pinpoint some of the shortcomings of the current understanding of the brain's involvement in the regulation of daily energy homeostasis.

For over a century now, increasingly sophisticated methods have been brought to bear on the topic of brain involvement in energy homeostasis. A vast number of experimental observations have been produced, and, particularly within the last decade, the combination of molecular genetic and physiological techniques has allowed for great progress in identifying metabolic hormones and establishing their relationship to key neuronal systems in the hypothalamus (Friedman and Halaas, 1998; Elmquist et al., 1999; Kalra et al., 1999; Schwartz et al., 2000; Seeley et al., 2004). The central integration of afferent signals that reflect both acute and chronic body energy requirements has become clearer. However, the nature and kinetics of neuronal responses that actually initiate the changes in ingestive behavior and metabolism are still largely unknown.

The involvement of various hypothalamic regions in the regulation of energy homeostasis was initially uncovered through a series of degeneration studies that revealed that the destruction of the hypothalamic ventromedial (VMH), paraventricular (PVH), and dorsomedial (DMH) nuclei induces hyperphagia (Anand and Brobeck, 1951; Brobeck, 1946; Hetherington and Ranson, 1940, 1942), whereas lesions in the lateral hypothalamus (LH) reduce food intake (Anand and Brobeck, 1951). While these studies may be considered crude by today's standards, they established the anatomical basis for modern research of energy regulation. In retrospect, considering our current knowledge of hypothalamic mechanisms of homeostasis, these lesion analyses

were strikingly precise in distinguishing between subregions of the hypothalamus that house circuits that either promote or inhibit feeding. On the other hand, these studies, in conjunction with physiological observations on animals that were obese (Coleman, 1982; Zucker and Zucker, 1963), suggested that humoral signals that arise from the peripheral tissues inform brain structures on the overall energy status of the body. The interaction between these two components defines the mechanisms of energy balance.

The discovery of leptin, the protein product of the *ob* gene, along with the unveiling of the melanocortin system have been fundamental in supporting such a view of energy homeostasis and in understanding the role of the mediobasal hypothalamus in energy regulation. Substantial advances have since been made to explain the molecular and cellular mechanisms by which leptin and other peripheral signals affect the melanocortin system. There also has been an increase in integrative approaches that link the functions of hypothalamic feeding circuits with those of systems that control ingestive behaviors and arousal. In this review, we aim to provide a synopsis of some of these advances.

### The Hypothalamic Melanocortin Model of Energy Balance

Subsequent to the discovery of the adipose hormone leptin (Zhang et al., 1994), the melanocortin model of energy regulation has held the most significance in understanding the neuronal control of energy homeostasis. In this model, the arcuate nucleus (ARC) is considered a critical region for various reasons: first, the neurons within the ARC are anatomically placed in close proximity to fenestrated capillaries at the base of the hypothalamus, giving them access to various humoral signals that have restricted entry to other portions of the brain (Benoit et al., 2000; Cone et al., 2001). These neurons are innervated by axons containing most, if not all, of the major neurotransmitters and expressing receptors for these neurotransmitters, as well as most metabolic hormones (Kalra et al., 1999; Benoit et al., 2000; Seeley et al., 2004; van den Pol, 2003). ARC cells also respond to the fluctuations of nutritional signals, including glucose, fatty acids, and insulin. These cells, therefore, were suggested to be the “first-order” sensory neurons (Schwartz et al., 2000; Obici and Rossetti, 2003). This nomenclature, however, may not be appropriate, as it implies an existence of proof of a hierarchical neuronal system in response to nutritional signals. The presence of receptors for nutritional signals in other hypothalamic nuclei, most notably in the VMH and LH, and their critical role in energy homeostasis argue that cells within the ARC are not the only ones receiving first-hand input from nutritional signals (Huang et al., 1996; Lynn et al., 1996; Mercer et al., 1996; Couce et al., 1997; Fei et al., 1997; Guan et al., 1997; Elmquist et al., 1998; Horvath et al., 1999, 2001; Shioda et al., 1998; Mitchell et al., 2001; Horvath and Gao, 2005; Zigman et al., 2006).

Two subsets of neurons were identified in the ARC; both contain the classical inhibitory neurotransmitter

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GABA (Horvath et al., 1997; Hentges et al., 2004). One of the populations, which when activated leads to a decrease in food intake and an increase in energy expenditure, expresses proopiomelanocortin (POMC) and cocaine-amphetamine-regulated transcript (CART) (Boston et al., 1997; Ellacott and Cone, 2004; Cone, 2005). The POMC precursor peptide is cleaved into melanocyte-stimulating hormones ( $\alpha$ -,  $\beta$ -, and  $\gamma$ -MSH), adrenocorticotrophic hormone (ACTH), and  $\beta$ -endorphin (Cone, 2005). Of these,  $\alpha$ - and  $\beta$ -MSH reduce body weight and food intake and increase energy expenditure in animals and humans (Fan et al., 1997; Biebermann et al., 2006; Lee et al., 2006) by acting on melanocortin receptor subtypes 3 and 4 (MC3 and -4) (Adan et al., 1994) that exist throughout the brain but are particularly abundant in the ARC, LH, DMH, and PVN (Mountjoy et al., 1994). In contrast, cells in which an increased activity leads to orexigenic response and reduced energy expenditure contain neuropeptide Y (NPY) and the agouti gene-related transcript (AgRP). NPY was found to potently stimulate food intake and reduce energy expenditure (Clark et al., 1984). In genetically obese animals, i.e., *ob/ob* and *db/db* mice, as well as animals in a negative energy state, i.e., fasted or lactating animals, a greatly increased ARC NPY mRNA and proteins were observed (Morley et al., 1987; Sahu et al., 1988a; Sancarora et al., 1990; Wilding et al., 1993). Although NPY is found throughout the brain (Sahu et al., 1988b), it is high in concentration in the ARC as well (Chronwall et al., 1984). A distinguishing feature of ARC NPY neurons is that they also contain AgRP, a natural antagonist of MC3 and -4 receptors, which, thereby, reduces the anorectic effect of  $\alpha$ -MSH (Ollmann et al., 1997; Quillan et al., 1998; Rossi et al., 1998; Tritos et al., 1998). An ectopic expression of Agouti, an AgRP-like peptide, results in the obese phenotype of the *yA* agouti mouse (Ollmann et al., 1997). Both POMC and NPY neurons in the ARC express leptin and ghrelin receptors (Dickson and Luckman, 1997; Elias et al., 1998, 2000; Baskin et al., 1999; Bailey et al., 2000; Hewson and Dickson, 2000; Riediger et al., 2003). Peripheral administration of either substance modulates the neuronal activity and gene expression of these neurons. Leptin increases the activity of POMC cells (Traebert et al., 2002) and inhibits that of NPY cells, while ghrelin does the opposite (Baskin et al., 1999). Ghrelin modulates POMC neuronal activity via activation of presynaptic GABA release (Cowley et al., 2001; Horvath et al., 1992).

It is important to note that most of our understanding of functional aspects of the melanocortin system is derived from gene transcription analysis, including the assessment of expression levels of neuropeptides, such as POMC and NPY, and early responding genes, such as *c-fos*, and the association between gene expression and feeding behavior. There is no direct evidence to conclude that increased firing of ARC NPY neurons in response to a changing metabolic milieu triggers appetite or that increased electrical activity of POMC neurons promotes satiety and cessation of feeding behavior. This is partially due to the lack of available experimental tools. The past years, however, have witnessed some remarkable advances due to new technologies. The neuron-selective expression of fluorescent reporter genes, for example, allows the visualization of hypothalamic

neuronal subpopulations so that they can be studied while the cells are alive. Through the use of this method, substantial neuroanatomical and electrophysiological data have been generated to demonstrate that NPY neurons contact nearby POMC cells and inhibit them through the release of GABA (Cowley et al., 2001). The unidirectional NPY to POMC input (Horvath et al., 1992) is an interesting feature of the anatomical basis of energy regulation because it may represent a wiring blueprint that favors the tonic inhibition of satiety signals, a feature that may have been derived through natural selection to not only promote feeding but also overfeeding in situations when food is available in excess (Bates and Myers, 2003).

The ARC POMC and NPY neurons project to various parts of the brain, which include the PVN, LH, and perifornical hypothalamic region that all contain substantial amounts of MC3 and -4 receptors (Mountjoy et al., 1994; Sahn et al., 1994). The projection from the ARC to the PVN is important for the regulation of neurons that produce corticotropin- and thyrotropin-releasing hormones (CRH and TRH, respectively) and for the modulation of sympathetic activation; both are significant physiological mechanisms in the regulation of metabolism (Cowley et al., 1999; Figure 1). Recently, an elegant study using a *cre/loxP* approach by Balthasar and colleagues showed that mice with mutations to the MC4 gene (*loxTB mc4*) weighed and ate less if the MC4 gene was selectively restored in the PVN via viral or genetic manipulations (Balthasar et al., 2005). However, the restoration of the MC4 receptors in the PVN was not sufficient to bring energy expenditure levels to those of controls, suggesting that energy expenditure is regulated by melanocortins elsewhere in the hypothalamus or through receptors other than MC4 receptor (Balthasar et al., 2005).

Two independent groups recently showed that intact melanocortin neuronal circuitry is necessary for acute regulation of feeding (Gropp et al., 2005; Luquet et al., 2005). The studies stemmed from the considerable frustration due to the fact that neither NPY nor AgRP single-gene-knockout mice (Erickson et al., 1996) nor NPY/AgRP double-knockout mice (Qian et al., 2002) exhibited the expected metabolic phenotypes. It could be argued that the classic knockout technology is inadequate for interpreting the acute adult mechanism, but a more reasonable question to ask is whether the NPY and POMC neurons themselves are crucial in adult energy regulation, whereas the loss of NPY/AgRP and/or POMC genes may be compensated for during development by, say, the classic neurotransmission system. The tool chosen by both groups was the cell-targeted expression of diphtheria toxin receptors (avian or human) that do not exist in mice, making these animals "immune" to diphtheria toxin-induced necrosis. When the otherwise normally developing diphtheria toxin receptor expressing mice were injected with two subsequent doses of diphtheria toxin, the targeted hypothalamic neurons rapidly died, resulting in hypophagia in NPY neuron-ablated mice (Gropp et al., 2005; Luquet et al., 2005) and hyperphagia in POMC neuron-ablated animals (Gropp et al., 2005). These studies provided direct proof for the argument that these subsets of ARC neurons are required in adult animals to regulate acute feeding behavior and body weight. Luquet et al. (2005) also found, however, that

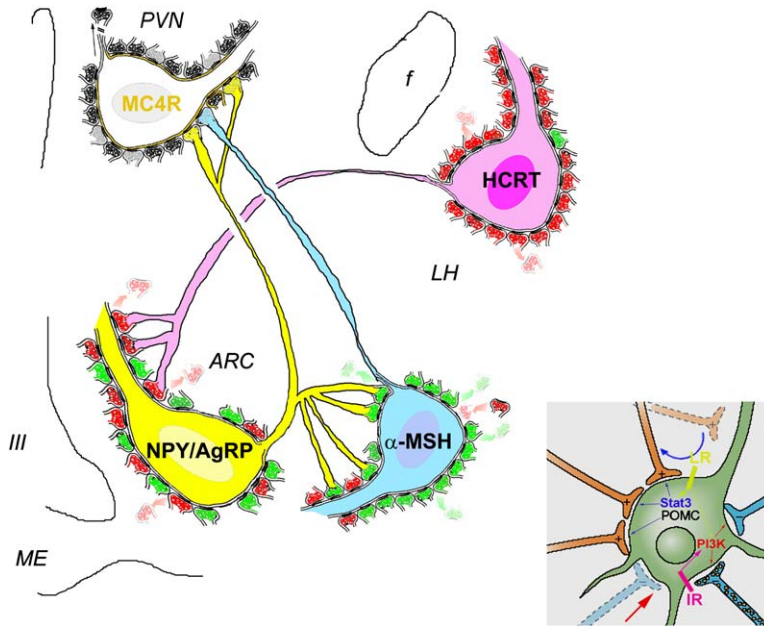


Figure 1. Schematic Illustration of the Melanocortin System

In the arcuate nucleus (ARC), the orexigenic NPY/AgRP neurons (yellow neuron) have an increased excitatory (red)/inhibitory (green) input ratio during negative energy balance (low leptin, high ghrelin levels in the circulation) compared to satiety. Arrows indicate the direction of synaptic movement after leptin levels increase and ghrelin levels diminish as satiety emerges. In contrast, neighboring anorexigenic  $\alpha$ -MSH cells (blue neuron) have an elevated inhibitory/excitatory input ratio during negative energy balance compared to satiety. These two systems have overlapping projections that target neurons that express melanocortin receptor 4 (MC4R) in various regions, including the PVN. MC4Rs (brown membranes in the PVN) may be localized postsynaptically or presynaptically in close proximity to AgRP- and  $\alpha$ -MSH-containing fibers. A proportion of the inhibitory inputs on melanocortin perikarya in the arcuate nucleus arise from local NPY/AgRP neurons. Some excitatory inputs on NPY/AgRP neurons contain hypocretin/orexin (neurons) originating in the lateral hypothalamus (LH). These lateral hypothalamic cells are also orexigenic, and

their perikarya are dominated by excitatory inputs both during negative energy balance and satiety, albeit the satiety level of excitatory inputs is somewhat blunted. All three of these cell types are frequently in close proximity to capillary vessels, thus increasing the likelihood of their direct regulation by circulating leptin or ghrelin. Components of the melanocortin system, as well as the hypocretin neurons, show synaptic plasticity in response to changing metabolic environment. These synaptic changes are leptin regulated (lower right panel), and hence may be triggered by intracellular signaling cascades of the leptin receptor (LRb). This includes Stat3 and PI 3-kinase signaling pathways. Because these signaling modalities can be initiated by other humoral signals as well (for example, insulin receptors [IR] activate PI3K) it is reasonable to anticipate that these synaptic changes are not limited to leptin's effect.

ablation of NPY neurons during the early postnatal period did not result in an overt metabolic phenotype, contending that functional effects of degenerated neurons in critical developmental periods may be overcome by reorganization or plasticity of the circuits. Interestingly, plasticity of hypothalamic feeding circuits remains in adults (see below and Figure 2).

### The Ventromedial Hypothalamus

The VMH was first implicated directly in the regulation of food intake and obesity when Hetherington and Ranson, as well as others, showed that electrolytic lesions to this region in rats resulted in rapid development of obesity (Hetherington and Ranson, 1940, 1942; Brobeck et al., 1943; Brobeck, 1946). A significant number of analyses that include chemical lesions as well as various pharmacological studies have since supported the notion that the VMH, like the ARC, is a critical hypothalamic nucleus that inhibits feeding and increases metabolism and, by doing so, restricts the amount of body fat (Bagnasco et al., 2002; Tejwani and Richard, 1986). In contrast, the destruction of the LH of rats resulted in profound anorectic states (Anand and Brobeck, 1951), suggesting that the LH is involved in promoting food intake. Together, these findings led to the development of a dual-center hypothesis of energy regulation, where the VMH was proposed to be a satiety center (Stellar, 1954) and the LH was described as a hunger center (Elmquist et al., 1999). It is, perhaps, not surprising that the VMH was later found to be one of a few key regions in the hypothalamus where the long-form leptin receptors, LRb, are highly expressed and, therefore, is

the region that mediates leptin's effect on homeostasis (Mercer et al., 1996; Fei et al., 1997). Despite the early discovery of the contribution of the VMH in feeding and metabolism and the involvement of leptin signaling in the VMH, little is known regarding the cellular

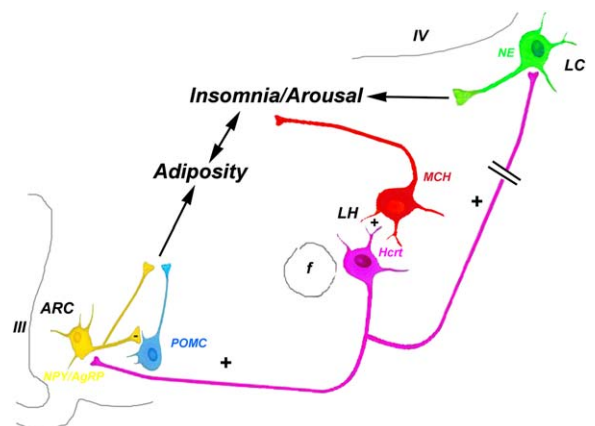


Figure 2. Schematic Illustration of an Anatomical Blueprint for Association between Insomnia and Obesity

The connectivity and synaptic input organization of lateral hypothalamic hypocretin neurons provide a simple and straightforward explanation for the relationship between insomnia and adiposity: because of the easy excitability of hypocretin neurons and their efferent connectivity, any signal that triggers their activity, regardless of the homeostatic needs, will elevate the orexigenic tone of local MCH neurons as well as the arcuate nucleus melanocortin system while also promoting wakefulness through activation of medullary locus coeruleus (LC) noradrenergic (NE) neurons.

mechanisms by which the VMH neurons regulate energy homeostasis under the control of leptin and other metabolic signals. Research interests shifted from the VMH to the ARC, particularly because of the discovery of the ARC NPY/AgRP and POMC/CART neuronal circuits (Broberger et al., 1998b; Elias et al., 1998; Hahn et al., 1998). This having been the predominant interest with publications that followed may have given the impression that the ARC melanocortin system was the primary component of the satiety center.

Recent studies, however, show that metabolic responses to leptin are only partially mediated by POMC cells in the ARC and that the VMH may play a more important role in the regulation of energy balance than previously thought (King, 2006). These studies examined the behavioral and metabolic phenotype of animals with the leptin receptor selectively deleted in POMC neurons (Balthasar et al., 2004). Surprisingly, these animals were at best only mildly obese and ate similar amounts of food as controls (Balthasar et al., 2004). In contrast, a selective deletion of the leptin receptor gene in the neurons that express steroid factor-1 (SF-1) in the VMH results in mice that are not only obese but also hyperphagic (Majdic et al., 2002; Dhillon et al., 2006). SF-1 is a transcription factor necessary for the development of the VMH (Parker et al., 2002; Davis et al., 2004; Segal et al., 2005), whereas, leptin increases the expression of SF-1. Mice with mutations to leptin receptors in both the ARC POMC and the VMH SF-1 neurons are more obese than those with mutations of leptin receptors in either set of neurons alone, suggesting that the VMH is just as sensitive to leptin as the ARC in energy regulation (Dhillon et al., 2006). This is not only consistent with the early discoveries obtained from the VMH lesion experiments but also helps explain why leptin simultaneously acts on both the ARC and VMH.

How, then, do VMH neurons act to deliver their anorectic effect in the context of hypothalamic feeding circuits? In a recent study, Sternson and colleagues showed that the VMH may increase the activity of POMC neurons via microcircuits (Sternson et al., 2005) that were previously difficult to detect by conventional tracing techniques (Saper et al., 1976; Zaborszky and Makara, 1979; Canteras et al., 1994). Using laser scanning photostimulation (LSPS) in combination with slice electrophysiology, these authors showed that the inputs from the VMH neurons are mostly excitatory, thus increasing the activity of POMC cells, and during a fast these inputs decrease (Sternson et al., 2005). They also confirmed the earlier observation by Pinto et al. (2004) that the input organization of the ARC POMC neurons varies depending on the metabolic state (Sternson et al., 2005). Whether these VMH neurons that provide plastic efferents onto POMC neurons receive leptin signaling requires further investigation. On the other hand, the ARC may also modulate the activity of the VMH. Afferent projections from the ARC to the VMH are sparser than those to other nuclei (Zaborszky and Makara, 1979), yet the VMH contains MC4 receptors as well as NPY Y1, Y2, and Y5 receptors (Bouali et al., 1995; Lopez-Valpuesta et al., 1996; Harrold et al., 1999; Wisialowski et al., 2000; Kishi et al., 2003; Li and Davidowa, 2004), suggesting that both POMC and NPY neurons project to the VMH. Infusions of NPY into the VMH increase feeding, and fasting is associated

with elevated levels of NPY in this region (Bouali et al., 1995; Kalra et al., 1999), whereas electrophysiological responses of neurons in the VMH to  $\alpha$ -MSH are decreased in animals that are fasted and/or treated with AgRP prior to sacrifice than in animals that have free access to food (Li and Davidowa, 2004).

A notable characteristic of the VMH is that it highly and specifically expresses the brain-derived neurotrophic factor (BDNF) that has been shown to affect the metabolic functions regulated by the VMH. Genetic deficiencies of BDNF or its TrkB receptor, on the other hand, resulted in obesity in both humans and mice (Rios et al., 2001; Xu et al., 2003). Interestingly, leptin increases BDNF transcripts (Nakagawa et al., 2002, 2003; Tsuchida et al., 2002; Komori et al., 2006), whereas fasting decreases BDNF transcripts selectively in the VMH, suggesting that the BDNF is, indeed, a regulatory component controlled by leptin signaling to control body energy balance. Since BDNF in the brain is known to promote synaptic morphology and function in various contexts (Rios et al., 2001), the specific effect and expression of BDNF in relation to homeostasis raises the possibility that synaptic plasticity in the hypothalamus may be a part of the regulatory mechanism of energy regulation. Notably, the anorectic effects of BDNF are not directly mediated by the melanocortin system since BDNF reduces the body weight and food intake of mice that lack the MC4 receptor (Xu et al., 2003), yet neurons in the VMH form excitatory microcircuits with POMC neurons in the ARC (Sternson et al., 2005), suggesting that the VMH may influence POMC neuroactivity by other means, such as classic neurotransmitters and/or plasticity. In fact, leptin and other metabolic hormones increase the number of excitatory synapses on POMC cells (see also "Soft Wiring of Circuits Associated with Feeding"). It is not yet known whether these excitatory inputs originate from the VMH.

### The Lateral Hypothalamus

As previously described, the LH was identified as the hunger center because its destruction in animals blocks feeding. Within the LH, two sets of neuron populations that contain either hypocretin (orexin), a peptide implicated in arousal and feeding, or melanin-concentrating hormone (MCH), another potent stimulator of food intake, have been identified. It was indicated that these neurons motivating food intake are within the confines of the ARC melanocortin system (Qu et al., 1996; de Lecea et al., 1998; Elias et al., 1998; Ludwig et al., 1998; Sakurai et al., 1998; Tritos et al., 1998; Dube et al., 1999; Edwards et al., 1999; Horvath et al., 1999; Saito et al., 2001; Tritos et al., 2001; Guan et al., 2002), albeit their position in the hierarchy is a subject of debate. Both MCH and hypocretin neurons have a wide projection field and modulate a variety of behavioral responses related to learning, memory, emotion, motivation, and motor responses in association with changes in the energy state (Broberger et al., 1998a; Ludwig et al., 1998; Peyron et al., 1998; Lu et al., 2000; Saito et al., 2001). Although the projections of MCH and hypocretin neurons exhibit significant overlap, their overall effects and actual targets are quite different (Broberger et al., 1998a; Horvath et al., 1999). Like that of the NPY and POMC neurons, the activity of MCH and hypocretin

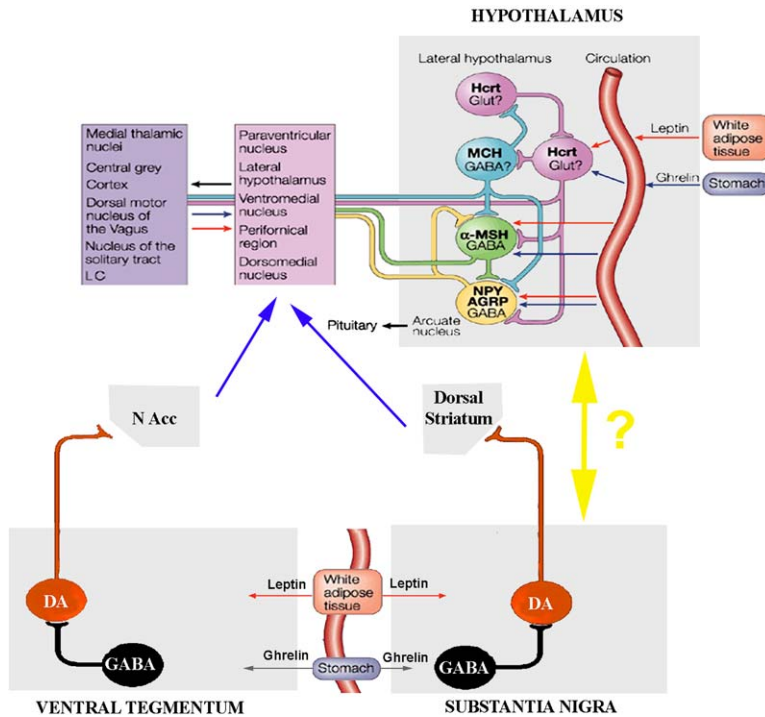


Figure 3. Relationship between Hypothalamic and Extrahypothalamic Regions in the Regulation of Energy Homeostasis

Schematic illustration depicting interactions and projections of several hypothalamic peptidergic systems, including orexin (ORX/Hcrt)- and melanin concentrating hormone (MCH)-producing neurons in the lateral hypothalamus as well as neuropeptide Y (NPY)/Agouti-related protein (AgRP)- and  $\alpha$ -melanocyte-stimulating hormone ( $\alpha$ -MSH)-producing neurons in the arcuate nucleus. In the hypothalamus, various peripheral hormones, including leptin and ghrelin, affect the release of the above mentioned neuromodulators. In the same fashion, extrahypothalamic circuits, for example, the mesencephalic dopamine system, are also targeted by peripheral hormone effects and alter behavioral (and potentially endocrine) components of energy homeostasis. Besides the mesolimbic dopamine reward system that consists of VTA dopamine neurons with projections to the nucleus accumbens (NAcc), recent data clearly indicate that the nigrostriatal dopamine system (originating in the substantia nigra and project to the dorsal striatum) is key in the regulation of feeding behavior. The precise signaling modality that places these mesencephalic dopamine systems up- and/or downstream to the hypothalamic machinery needs to be determined. DA, dopamine; GABA,  $\gamma$ -aminobutyric acid; Glut, glutamate.

neurons is regulated by numerous hormones that include leptin and ghrelin, as well as by practically every neurotransmitter system (Elias et al., 2000; Hakansson et al., 1998; Torrealba et al., 2003; Toshinai et al., 2003; Sakurai et al., 2005). Within the LH, MCH and hypocretin neurons have reciprocal connections with each other and with nearby neurons (Guan et al., 2002). Electrophysiological studies in brain slices or isolated neurons indicated that hypocretin, in general, has a stimulative effect on LH neurons, including MCH neurons (van den Pol et al., 2004), whereas MCH depresses the synaptic activity of glutamate and GABA neurons from the rat LH (Gao and van den Pol, 2001). It is not clear whether such electrical interactions between hypocretin and MCH neurons, at the various intensities and dynamics observed, are relevant to feeding and long-term homeostasis or whether they are more related to arousal behavior, and this will be interesting to elucidate.

Like the neurons in the ARC, MCH and hypocretin neurons are capable of directly integrating metabolic signals to modulate energy balance, and they do so, more or less, independent of each other and despite their proximate location and physiological interaction in the LH. For example, mRNA levels of hypocretin in the LH are upregulated upon fasting (Sakurai et al., 1998). Notably, hypocretin neurons are rapidly activated by fasting in rodents and nonhuman primates (Diano et al., 2003) and exhibit leptin-dependent synaptic plasticity during fasting (Horvath and Gao, 2005). Moreover, all of the hypothalamic neurons that are activated by fasting receive strong hypocretin input. These observations, together with the earlier demonstration of a massive hypocretin input to the ARC, particularly to the

NPY neurons (Horvath et al., 1999), as well as the dependence of hypocretin function on NPY signaling (in particular on the NPY Y1 and Y5 receptors) (Yamanaka et al., 2000), and a synergistic action between NPY and hypocretin (at low concentrations) to induce feeding (Sahu, 2002) argue that hypocretin neurons may be upstream to the NPY system with regard to feeding and energy metabolism. Mice with deletions of the hypocretin gene are hypophagic but maintain normal growth curves, suggesting that they possess a reduced metabolic rate (Hara et al., 2001; Willie et al., 2003). This, in association with the critical role of hypocretin neurons to promote arousal (Chemelli et al., 1999) through direct projections to the brain stem, including the locus coeruleus (Horvath et al., 1999), makes the hypocretin neurons a likely candidate for providing the link between obesity and insomnia (Figure 3). However, whether this circuitry has a real functional role in coordinating sleep/wake cycles with energy metabolism needs further experimental support, particularly in light of the observations that genetic manipulation of the hypocretin neuronal system in mice does not support the notion that these neurons are critical for feeding.

The orexigenic peptide MCH, on the other hand, shows no or little interaction with NPY or hypocretin in inducing food intake when injected together into the third ventricle of the rat (Sahu, 2002). These observations provide a physiological component concomitant to the preexisting morphological data that shows that the strength of MCH projections to the ARC is limited compared to that of the hypocretin neurons. Thus, the action of MCH on feeding is likely independent of NPY and hypocretin action. However, like NPY, MCH does

exhibit characteristics typical of orexigenic genes: its mRNA levels are increased in obese mutant animals, and fasting further increases its expression in both normal and obese animals (Qu et al., 1996), and it has a potent orexigenic effect. Targeted deletion of the MCH gene, on the other hand, results in a phenotype of hypophagia and leanness with an inappropriately high metabolic rate (Shimada et al., 1998), suggesting that MCH is a typical “thrifty gene” that increases energy intake and reduces energy expenditure simultaneously. Consistent with this idea, MCH suppresses thyroid-stimulating hormone (TSH) release (Kennedy et al., 2001). In contrast to hypocretin that acts through NPY, MCH appears to compete with the action of  $\alpha$ -MSH to produce its effect (a mechanism conserved from skin color regulation in fish to hypothalamic control of energy balance in mammals), such that MCH administration increases feeding, while  $\alpha$ -MSH acts to decrease it. When the peptides are administered together, depending on the relative dose, one antagonizes the action of the other (Ludwig et al., 1998). Recently, a mouse model of MCH neuron ablation was generated by expressing a toxin gene, ataxin-3, targeted at MCH neurons (Alon and Friedman, 2006). Mice that express this gene have a chronic loss of MCH neurons. Interestingly, the phenotype of these mice highly resembles that of mice lacking only the MCH gene, that is they exhibit reduced food intake and increased energy expenditure. Moreover, the ablation of MCH neurons in mice with an *ob/ob* mutant background resulted in improved obesity and glucose tolerance. The results of neuron-ablation studies, however, suggest that the function of MCH cells in energy regulation may be limited to the MCH system itself, and not to other aspects of the cells, such as their classic neurotransmitter function and/or their synaptic plasticity, which are distinct from NPY cells (see below).

#### Other Systems Associated with Functional Correlates of the Melanocortin System

Receptors for leptin and ghrelin are also found in other hypothalamic nuclei, including the DMH and the supra-chiasmatic nucleus (SCN), which supports the idea that these hormones directly target those nuclei to generate additional behavioral and physiological responses that complement those mediated by cells in the ARC (Hakansson et al., 1998; Zigman et al., 2006). The DMH has, for some time, been implicated in the regulation of energy balance, yet its actual role remained obscure until recently (Bellinger and Bernardis, 2002; Chou et al., 2003; Gooley et al., 2006). The DMH is involved in a variety of regulatory mechanisms that include the modulation of glucocorticoid secretion, body temperature, arousal, and circadian rhythms of locomotor activity (Chou et al., 2003). The DMH receives inputs from cells in the ARC and from brain stem centers that are also implicated in feeding regulation (Bellinger and Bernardis, 2002). Lesions restricted to the DMH typically result in hypophagia, although animals can still maintain their body composition (Bellinger and Bernardis, 2002). In a recent study, Gooley et al. showed that the DMH is critical for the entrainment of circadian rhythms to feeding schedules (Gooley et al., 2006). As they and others showed, the DMH of animals with restricted access to food (4 hr/day) had increased expression of c-Fos, indicating

an increased cellular activation at a time when the food was regularly presented compared to animals that had free access to food throughout the day. Ibotenic lesions of the DMH resulted in reduced levels of locomotor activity and decreased food intake. Furthermore, when lesioned rats were placed in the restricted feeding schedule, they showed less preprandial increases in food anticipatory locomotor activity than those of sham-operated animals. DMH lesions also blocked the rise in body temperature that is entrained to the timing of food presentation (Gooley et al., 2006). The phenotype of cells within the DMH remains obscure. A number of these cells produce glutamate as a neurotransmitter and project to the PVN as well as to the preoptic area, both thought to be involved in the circadian regulation of corticosteroid secretion and body temperature (ter Horst and Luiten, 1986; Bellinger and Bernardis, 2002). Projections from the DMH to the LH and to the ventrolateral preoptic area have been implicated in sleep and arousal and could presumably relate to the enhanced activity of animals in restricted feeding schedules (Chou et al., 2002).

The role of the SCN in regulating energy balance has been overshadowed by the critical role of this hypothalamic region as a master clock that mediates circadian patterns of biological function (Morin and Allen, 2005). Among these, the SCN controls the circadian secretion of metabolic hormones and is thought to regulate seasonal adipose tissue content and maintain patterns of glucose levels (Buijs et al., 2003; Sumova et al., 2004). The SCN projects to most hypothalamic nuclei, with a strong projection field that terminates in the PVN and DMH (Morin and Allen, 2005). Recent evidence suggests that the ARC projects to the SCN as well, indicating that the melanocortin system can influence its activity (Yi et al., 2006). Also, mice with mutations of the *clock* gene, a transcript expressed in the SCN and critical to the transcriptional mechanisms important in the generation of circadian rhythms, exhibit an obese phenotype (Turek et al., 2005). Hypothalamic *clock* gene expression is highest in the SCN, thus directly implicating this region in obesity caused by this mutation (Hastings et al., 2003; Maywood et al., 2003). However, *clock* mRNA is also present in the ARC and median eminence, suggesting that the *clock* gene could modulate metabolic function via cells in these regions (Abe et al., 2001).

Other brain circuits also exist that, when activated, modulate food intake and body weight. Although these systems cannot be fully considered homeostatic, activation of these pathways could override regulatory signals from hypothalamic homeostatic centers to either increase or decrease appetite (Berthoud, 2002). For example, it is well established that rats whose brain stem is isolated continue to regulate the food they consume and show affective responses to palatable foods (Grill and Kaplan, 2001, 2002). Another example is the corticolimbic pathways that are capable of integrating sensory inputs to produce cognitive representations that are stored and used for making decisions. Lesions to various corticolimbic regions result in obesity (Clifton et al., 1998; Rollins et al., 2001).

One system that is emerging as a “player” in the regulation of energy homeostasis is the mesencephalic dopamine system. Dopamine is involved in the

regulation of arousal, locomotor activity, mood, and reward (Wise, 2002). Dopamine deficiency in mice, generated by selective inactivation of tyrosine hydroxylase, markedly suppresses food intake in a manner that is similar to that of lesions of the LH (Szczyepka et al., 1999a, 1999b). These mice fail to eat in response to acute glucose deprivation (Hnasko et al., 2004), as well as to PYY administration or leptin deficiency (Szczyepka et al., 2000), suggesting that there is an absolute requirement for dopamine signaling that appears to act downstream of the melanocortin system for promoting feeding. With regard to the various functions of the dopamine system, reward pathways have received particular attention in explaining feeding behavior given the universality of food as a natural reinforcer. Dopaminergic neurons within the midbrain ventral tegmental area (VTA) that innervate the nucleus accumbens (the ventral striatum) have been implicated in the rewarding aspects of food, sex, and drugs of abuse (Volkow and Wise, 2005). In support of a role for the mesolimbic reward circuitry in feeding regulation, it was recently found that interfering with ghrelin signaling specifically in the VTA diminished ghrelin-induced feeding (Abizaid et al., 2006). Surprisingly, however, the restoration of dopamine production within the dorsal striatum restores feeding on normal chow, whereas restoration of dopamine in the nucleus accumbens does not. While these findings may suggest a fundamental difference between feeding for nourishment and food as a rewarding substance (Szczyepka et al., 2001), a similar study in human subjects argues in the same vein: by using positron emission tomography (PET) scanning, it was found that feeding is associated with dopamine release in the dorsal but not the ventral striatum, and yet the amount of dopamine released correlated with the degree of pleasure experienced (Small et al., 2003). Thus, further investigations are needed to discern the role of VTA versus nigral dopamine neurons in the regulation of feeding and energy homeostasis in general (Cannon and Palmiter, 2003; Sotak et al., 2005).

Nevertheless, feeding is associated with motivational mechanisms, which are important for the behavioral responses necessary for seeking food (Berridge, 1996). Hypothalamic peptides like NPY,  $\alpha$ -MSH, AgRP, hypocretin, and MCH, on the other hand, modulate the activity of dopaminergic neurons that target the nucleus accumbens (Berthoud, 2002). The ARC funnels metabolic information from signals like leptin to modulate the activity of the mesolimbic dopaminergic system via direct projections to the nucleus accumbens or indirectly through the activation of hypocretin or MCH neurons that also project to both the VTA and nucleus accumbens (Berthoud, 2002). Emerging evidence, however, supports the notion that at least the VTA is sensitive to leptin, insulin, and ghrelin and that the activity of dopaminergic neurons within the VTA can be modulated by these signals (Abizaid et al., 2006; Jerlhag et al., 2006; Wellman et al., 2005; Guan et al., 1997; Figlewicz, 2003; Zigman et al., 2006). The implications of these observations and their interpretation remain controversial. Further research may reveal that, in contrast to the funnel hypothesis, metabolic signals could act directly on reward systems, including dorsal striatum, to modulate motivational aspects of feeding in tandem with homeo-

static systems (Fulton et al., 2000; Figlewicz, 2003), although, perhaps, in a more sophisticated way.

#### Soft Wiring of Circuits Associated with Feeding

Cajal's neuronal doctrine predicts that the output of a neuronal population is greatly influenced by its input organization. Thus, the inquiry into whether various metabolic states might correlate with different organizations of synaptic input on the melanocortin cells was pursued. This concept raised the possibility that metabolic signals, leptin and ghrelin in particular, may have acute effects on synaptic plasticity within the appetite center. Indeed, the hypothalamus has been known to retain some form of plasticity in adulthood. For example, the rearrangement of synapses has been shown to occur in the magnocellular system during changes in water homeostasis (Miyata et al., 1994; Stern and Armstrong, 1998; Theodosis et al., 2004), in the arcuate nucleus interneuronal system during changes in the gonadal steroid milieu (Garcia-Segura et al., 1994; Parducz et al., 2002; Parducz et al., 1996), and on the perikarya of luteinizing hormone-releasing hormone neurons during changes in the gonadal steroid milieu (Zsarnovszky et al., 2001) or in photoperiod lengths (Xiong et al., 1997). Such synaptic plasticity has not been considered previously as an important aspect in the regulation of daily energy control. Observations by several groups (Pinto et al., 2004; Horvath and Diano, 2004; Horvath and Gao, 2005; Sternson et al., 2005) now suggest that it may be a regulatory component in the physiological environment and that under pathological conditions the synaptic constellation and its plasticity may be impaired. First, results on leptin replacement in *ob/ob* mice raised (Pinto et al., 2004) the question whether the observed synaptic rearrangements of feeding circuits is part of more general phenomena. This appears to be the case, as robust effects of peripheral ghrelin injections were detected on POMC neurons of mice, leading to a wiring different from that induced by leptin and in line with ghrelin's orexigenic action (Pinto et al., 2004). In addition, rapid, leptin-dependent rearrangement of the input organization of hypocretin neurons was also reported (Horvath and Gao, 2005), and recently we found that the effect of ghrelin on the midbrain dopamine system is also associated with rapid synaptic remodeling (Abizaid et al., 2006).

An important issue is whether these synaptic alterations have an impact on neuronal activity and, consequently, on the metabolic phenotype. While this remains to be proven, a task that is still daunting even for long-term potentiation and long-term depression (Malenka and Bear, 2004), it is important to recognize that these changes occur both preceding and concomitant to the varying behavioral and endocrine outputs (Pinto et al., 2004; Horvath and Gao, 2005). Even if this type of synaptic plasticity doesn't directly affect action potentials, the changing input organization of neuronal perikarya should have an impact on the set point of these cells under various circumstances (Horvath and Diano, 2004; Horvath and Gao, 2005). For example, the probability that a *trans*-synaptic excitatory signal will trigger an action potential is high if this input is located proximally with fewer surrounding inhibitory connections versus more. If synaptic wiring does participate in determining

the set point of the central feeding circuits, rather than acutely determining postsynaptic activity, then subjects who are sensitive to diet-induced obesity could have a differential wiring and plasticity of hypothalamic and extrahypothalamic systems than those who are resistant, even before obesity would develop. If this is the case, then the argument could be made that developmental programming of hypothalamic circuits contributes not to obesity per se but rather to predisposing individuals to the likelihood of developing obesity later in life. Because of the easily measurable output of feeding circuits (amount of food consumed) that do not rely on animal training, we suggest that hypothalamic models of synaptic plasticity may offer a more effective avenue to provide proof for a causal relationship between synaptic plasticity and behavior associated with a circuitry.

### Concluding Remarks

Since the discovery of leptin, much has been added to our understanding of the intricate pathways through which metabolic signals target hypothalamic centers to alter energy balance. These advances, however, have come in the face of a rising obesity pandemic that has reached alarming proportions in the general population. The numbers are so staggering that some have predicted a decline in life expectancy within the next 75 years (Olshansky et al., 2005). Its economic impact is also distressing. Within the United States, the direct and indirect costs of obesity are estimated to be more than 120 billion dollars, an amount that is comparable to diseases associated with cigarette smoking and one that could pay the external debt of many underdeveloped countries (Stein and Colditz, 2004). The path that is necessary to offset this trend is not easy. There are a number of complicating factors. The human species as well as most others have evolved a genetic blueprint through natural selection that promotes energy storage for times in which energy may not be readily available from the environment. This particular set of "thrifty genes" in combination with the more sedentary lifestyle that has become the norm for western societies have turned these adaptive genes against us. While it is obvious that reducing caloric intake and increasing physical activity provide the answer to preventing and/or curing obesity and its associated disorders, it is unlikely to provide the short-term solution. It will be difficult to change the fact that average people in an urban setting spend most of their waking hours sitting down. Thus, the more we understand the neurobiological basis of feeding behavior and energy expenditure, the greater the likelihood for the development of pharmacological intervention. That, however, cannot be accomplished without a continued adaptation of state-of-the-art neurobiological approaches and philosophy for the study of energy metabolism.

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