

## Review

# Arcuate Nucleus-Dependent Regulation of Metabolism – Pathways to Obesity and Diabetes Mellitus

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**Abbreviations:** 5-HT, 5-hydroxytryptamine, serotonin; 5-HT2CR, 5-hydroxytryptamine receptor 2C; AgRP, agouti-related peptide; ARC, arcuate nucleus of the hypothalamus; BNST, bed nucleus of the stria terminalis; CCK, cholecystokinin; CGRP, calcitonin gene-related peptide; CNS, central nervous system; DMH, dorsomedial hypothalamus; ER, endoplasmic reticulum; GABA,  $\gamma$ -aminobutyric acid; GHSR, growth hormone secretagogue receptor; GLP1-R, glucagon-like peptide-1 receptor; HFD, high fat diet; IR, insulin receptor; LEPR, leptin receptor; LH, lateral hypothalamus; LPBN, lateral parabrachial nucleus; MC3R, melanocortin 3 receptor; MC4R, melanocortin 4 receptor; MCH, melanin concentrating hormone; ME, median eminence; NPY, neuropeptide Y; NTS, nucleus of the solitary tract; OXTR, oxytocin receptor; PBN, parabrachial nucleus; PNOC, prepronociceptin; POMC, pro-opiomelanocortin; PVH, paraventricular nucleus of the hypothalamus; PVT, paraventricular thalamus; PYY, peptide YY; SIRT1, sirtuin 1; STAT3, signal transducer and activator of transcription 3; TCPTP, T-cell protein tyrosine phosphatase; Vglut2, vesicular glutamate transporter 2; Xbp1, X-box binding protein 1.

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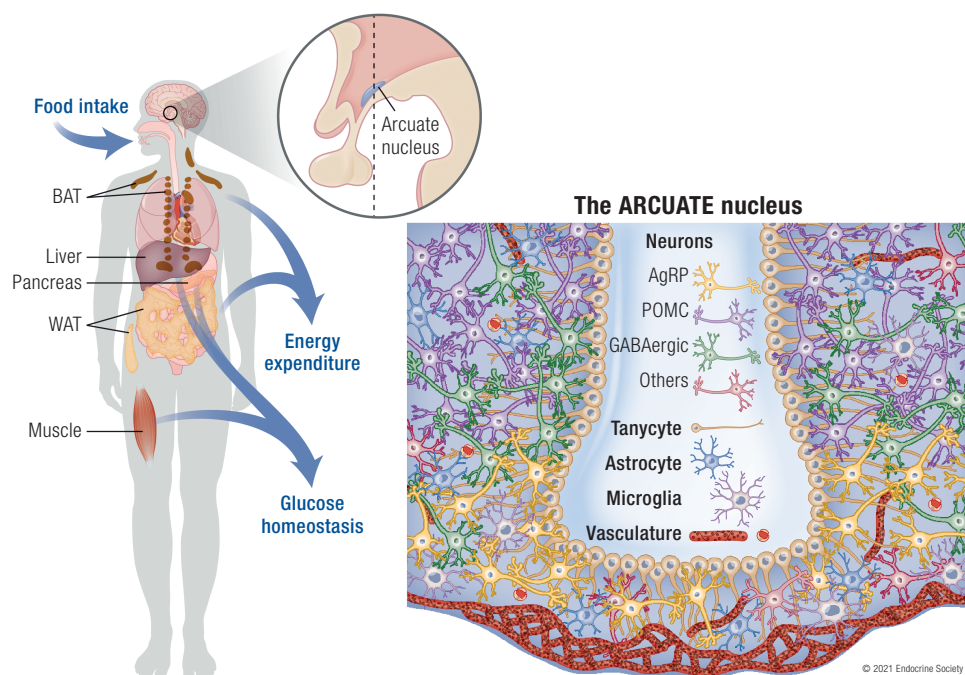
## Abstract

The central nervous system (CNS) receives information from afferent neurons, circulating hormones, and absorbed nutrients and integrates this information to orchestrate the actions of the neuroendocrine and autonomic nervous systems in maintaining systemic metabolic homeostasis. Particularly the arcuate nucleus of the hypothalamus (ARC) is of pivotal importance for primary sensing of adiposity signals, such as leptin and insulin, and circulating nutrients, such as glucose. Importantly, energy state-sensing neurons in the ARC not only regulate feeding but at the same time control multiple physiological functions, such as glucose homeostasis, blood pressure, and innate immune responses. These findings have defined them as master regulators, which adapt integrative physiology to the energy state of the organism. The disruption of this fine-tuned control leads

to an imbalance between energy intake and expenditure as well as deregulation of peripheral metabolism. Improving our understanding of the cellular, molecular, and functional basis of this regulatory principle in the CNS could set the stage for developing novel therapeutic strategies for the treatment of obesity and metabolic syndrome. In this review, we summarize novel insights with a particular emphasis on ARC neurocircuitries regulating food intake and glucose homeostasis and sensing factors that inform the brain of the organismal energy status.

**Key Words:** hypothalamus, arcuate nucleus, energy homeostasis, feeding, obesity, type 2 diabetes mellitus

## Graphical Abstract



### ESSENTIAL POINTS

- The arcuate nucleus of the hypothalamus is an integrator of nutrient-state communicating signals.
- Melanocortin neurons in the arcuate nucleus receive and integrate this information to implement appropriate behavioral and metabolic responses in order to maintain energy and metabolic homeostasis.
- Disruption of this fine-tuned control leads to an imbalance between energy intake and expenditure as well as deregulation of peripheral metabolism.

Obesity prevalence has increased worldwide in the last 50 years to pandemic proportions (1). Obesity is associated with a number of chronic diseases, such as type 2 diabetes mellitus, hypertension, dyslipidemia, cardiovascular diseases, certain cancer types, and neurodegenerative disorders (2, 3), which impose a significant socioeconomic burden on our society. Individuals with obesity are often

perceived as lacking will power and self-discipline to reduce excess energy intake and to increase physical activity and are vulnerable to social stigma and discrimination (4).

Biological and clinical evidence reveals a complex interaction of genes and environmental factors that is at odds with the assumption that body weight is entirely under voluntary control. In this context, the central nervous

system (CNS) plays a pivotal role in sensing and controlling metabolic homeostasis of the organism. Genome-wide association studies find that a majority of genes associated with body mass index are expressed in the CNS, many of them in the hypothalamus (5-7). Circulating nutrients, metabolites, and hormones, released by peripheral organs such as the adipose tissue, liver, pancreas, and the gastrointestinal tract, act as homeostatic feedback signals to the brain, which implements appropriate behavioral and metabolic responses in order to maintain energy and metabolic homeostasis. Interactions of distinct neuronal populations embedded in complex neuronal circuits monitor the internal state and provide tight homeostatic control of not only food intake and body weight, but also glucose metabolism, thirst, and blood pressure. Here, several types of neurons in various hypothalamic nuclei regulate autonomic and behavioral responses in relation to energy and glucose homeostasis. Probably the region studied in greatest detail comprises the arcuate nucleus of the hypothalamus (ARC). In this review, we will highlight recent conceptual advances in our understanding of how the ARC senses nutritional signals and integrates this information to control eating behavior, energy expenditure, as well as systemic glucose homeostasis, and how disruption of such regulation leads to obesity and associated metabolic diseases.

### The Arcuate Nucleus of the Hypothalamus: an Integrator of Nutrient-State Communicating Signals

A considerable amount of evidence points to a pivotal role for the ARC in metabolic control. Owing to its privileged anatomical location in the immediate vicinity to the median eminence (ME), this region is ideally positioned to receive the multifaceted feedback signals from the periphery of the organism. Classically, the control of metabolism and feeding regulatory neurocircuits has been viewed to be homeostatically controlled via feedback signals originating from the periphery of the organism to communicate its energy state (8). Here we provide an overview of such signals and the mechanisms that regulate their access to the CNS.

### Specialized Transport Mechanisms for Energy State Communicating Signals to the Arcuate Nucleus of the Hypothalamus

Nutritional feedback signals reach the hypothalamus in part via an incomplete, fenestrated endothelium in the ME, which plays a significant role in the actions of energy-state-sensing signals (9, 10). The brain vasculature is generally characterized by a blood-brain barrier that separates internal environments between the blood and brain parenchyma. However,

the fenestrated capillaries of the ME allow for the transport of peripheral signals into the nutrient-sensing hypothalamic nuclei (11, 12). Moreover, tanycytes, a specialized ependymal cell type that contacts the cerebrospinal fluid in the third ventricle, form a barrier and actively transport circulating molecules from the ME to the ARC (13). A decrease in blood glucose levels during fasting alters the structural organization of the blood-hypothalamus barrier in a vascular endothelial growth factor-dependent manner, thereby modulating the access of blood-borne metabolic substrates to the ventromedial ARC (13). For example, tanycytes have been shown to contribute to the entry of leptin into the hypothalamus and this transport function is altered in high-fat diet (HFD)-fed mice (14). The ablation of tanycytes of the ARC and ME leads to increased susceptibility to obesity, yet does not alter leptin sensitivity (15). Similarly, recent studies have questioned leptin receptor expression in tanycytes, and inactivation of the leptin receptor gene in tanycytes did not result in an overt metabolic phenotype (16). Collectively, while tanycytes exert a clear regulatory function in control of ARC neurocircuitries, the specific role and mechanisms of tanycyte-dependent regulation of leptin action awaits further clarifications. Interestingly, this barrier function seems to be, at least in part, under neuronal control as melanin-concentrating hormone (MCH)-expressing neurons, located in the lateral hypothalamus (LH), provide dense projections to the ME and regulate the permeability of the ME barrier via MCH-neuron-derived vascular endothelial growth factor (17). Thus, state-dependent regulation of ME barrier permeability represents an important novel regulatory hub in homeostatic control of feeding and metabolism. Here, the largely unexplored cellular heterogeneity of tanycytes and other cellular components of the ME barrier and their specific role in metabolic homeostasis clearly offers space for future investigation.

### Melanocortin Neurons in the Arcuate Nucleus of the Hypothalamus

Two well-characterized, interrelated, and functionally antagonistic neuronal populations in the ARC coordinately regulate appetite and homeostatic feeding. The orexigenic agouti-related peptide/neuropeptide Y-coexpressing (AgRP/NPY) neurons and the anorexigenic pro-opiomelanocortin/cocaine-amphetamine related transcript (POMC/CART)-coexpressing neurons. These neurons modulate the activity of a wide-ranging network of postsynaptic melanocortin 4 receptor (MC4R)-expressing neurons, of which particularly the ones located in the paraventricular nucleus (PVH) play an important role in regulating energy balance (18, 19). Accordingly, chemogenetic activation of MC4R-expressing neurons in the PVH reduces food intake after

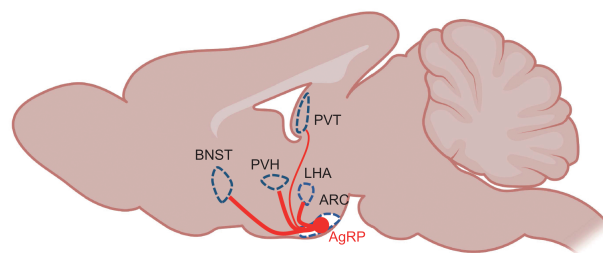
fasting, indicating that activation of these neurons is sufficient to promote satiety (20). The important evolutionary role for the melanocortin-dependent control of feeding is further highlighted by the fact that MC4R mutations are the most common cause of monogenic forms of obesity and are associated with early-onset severe obesity in humans (21, 22).

### Agouti-related Peptide-expressing Neurons

Agouti-related peptide (AgRP) is a neuropeptide that acts as an inverse agonist for melanocortin receptor 3 (MC3R) and MC4R (23). AgRP neurons are located at the bottom of the third ventricle close to the ME, which particularly allows them to sense and integrate peripheral metabolic signals. Energy deficit increases AgRP neuronal excitability, which is rapidly suppressed on the initiation of feeding (24–26). These neurons also release NPY as well as the inhibitory neurotransmitter  $\gamma$ -aminobutyric acid (GABA), and activation of AgRP neurons to promote feeding depends both on NPY and GABA release from these cells (27, 28). NPY is required for the short-term acute effects of AgRP neurons on feeding behavior as NPY-deficient mice fail to rapidly increase food intake during either chemogenetic or optogenetic activation of AgRP neurons (29). Moreover, another study revealed an important function for NPY release to mediate the food intake-stimulating effect of optogenetic AgRP-neuron prestimulation before refeeding (30). Nevertheless, the synaptic mechanisms involved in the AgRP-neuron stimulation-dependent release of NPY in feeding regulation are still subject to investigation, and the mechanisms as to how NPY released from AgRP neurons modulates the feeding and glucose metabolism regulatory effects on AgRP neuron activation still await further detailed clarification.

Moreover, the activity of AgRP neurons is required for compensatory refeeding after food deprivation (31). GABA-releasing AgRP terminals directly inhibit POMC neurons, thereby exerting a dual inhibition on POMC signaling (32, 33). AgRP neurons project to the PVH, and optogenetic activation of these projections has been shown to induce feeding (32, 34). Furthermore, optogenetic stimulation of AgRP projections to the LH, the anterior bed nucleus of the stria terminalis (BNST), and the paraventricular thalamus evoked a feeding response (35) (Fig. 1).

The overall importance of these neurons in feeding regulation is clearly evidenced by the phenotype of mice with diphtheria toxin-induced ablation of AgRP neurons in adult mice. Diphtheria toxin-induced ablation in adult mice leads to a strong reduction in food intake and eventually to starvation (36, 37). Interestingly, ablation of AgRP



**Figure 1.** Photostimulation of agouti-related peptide (AgRP) neuron axon projections increases food intake. Schematic illustration of a sagittal brain section showing AgRP projection fields that increase food intake on photostimulation. Photostimulation of inhibitory AgRP projections to the anterior bed nucleus of the stria terminalis (BNST), lateral hypothalamus (LHA), and the paraventricular nucleus of the hypothalamus (PVH) evokes a profound feeding response. Activation of AgRP → paraventricular thalamic nucleus (PVT) projections induced a moderate level of feeding. However, photostimulation of AgRP projections to the amygdala and the hindbrain did not increase feeding (35). Projection trajectories are schematic. The illustration of the sagittal brain section was created with BioRender.com.

neurons in neonates had only minor effects on feeding behavior, suggesting a window of plasticity, where developmental lack of AgRP neurons can be compensated for (37). In adult mice with ablated AgRP neurons the chronic delivery of a GABA(A) receptor partial agonist into the parabrachial nucleus (PBN) is sufficient to restore physiological feeding behavior (38). This finding emphasizes the importance of GABAergic output to the PBN in feeding regulation.

Besides the role in control of feeding, activation of AgRP neurons leads to a rapid switch in substrate use, decreasing fat use, and increasing systemic glucose use, independent of food intake (39). In addition to regulation of whole-body substrate use, AgRP neurons also exert a glucose-regulatory function via regulation of peripheral insulin sensitivity. Acute activation of AgRP neurons causes impaired systemic insulin sensitivity (29, 34). Here, these neurons control systemic insulin sensitivity via acute reprogramming of brown adipose tissue gene expression toward a myogenic signature, increasing myostatin levels and thereby coordinating hunger states with glucose homeostasis (34). This AgRP-neuron-dependent induction of peripheral insulin resistance results in reduced peripheral glucose deposition when food sources are limited.

Furthermore, the activity of AgRP neurons abrogates inflammatory pain, indicating an antinociceptive effect of hunger and the prioritization of survival needs (40). In addition, AgRP stimulation is mildly aversive, which suggests that AgRP neurons are responsible for negative valence signals produced during hunger (25). Overall, these data suggest a complex feeding-state-dependent role of AgRP neurons in control of peripheral substrate use and glucose



metabolism, and may point to different timings of acute regulation of insulin sensitivity versus a more prolonged regulation of substrate use.

Insulin receptors (IRs) are widely expressed in the CNS, and the brain-specific disruption of the IR leads to the development of obesity with increases in body fat and leptin levels, mild insulin resistance, elevated insulin levels, and hypertriglyceridemia (41). Central players for insulin action in the CNS are AgRP neurons and here, insulin inhibits AgRP neuron firing via IR-dependent signaling, which leads to a suppression of hepatic glucose production during euglycemic-hyperinsulinemic clamp studies (42).

Hunger, a state of negative energy balance, increases circulating ghrelin levels (43-45). This suggests that ghrelin acts as a preprandial meal initiation signal. Consistent with this concept, ghrelin induces feeding by potently stimulating AgRP/NPY neuronal activity. AgRP/NPY knockout mice do not increase food intake in response to ghrelin, indicating that AgRP/NPY neurons are required for the orexigenic effects of ghrelin (46, 47). AgRP neuron-selective expression of the ghrelin receptor growth hormone secretagogue receptor (GHSR) partially restores the orexigenic response to administered ghrelin in otherwise GHSR-null mice and fully restores the lowered blood glucose levels observed on caloric restriction in GHSR-null mice (48). Conversely, AgRP neuron-selective GHSR deletion abolishes ghrelin's acute orexigenic effects (49).

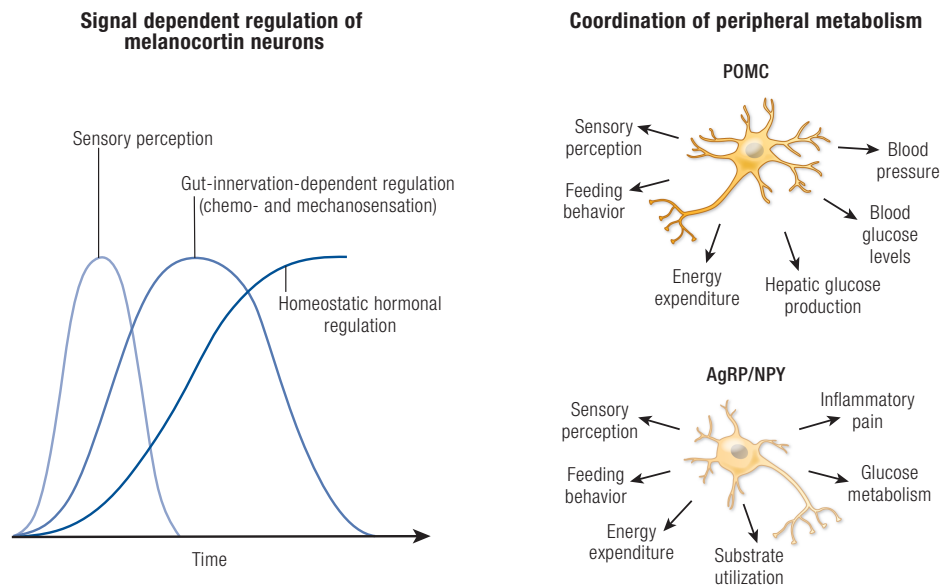
Furthermore, cholecystokinin (CCK) peptides are known to be released from enteroendocrine cells (50) and cerebral neurons, specifically a subset of neurons in the nucleus of the solitary tract (NTS). Activation of these CCK<sup>NTS</sup> neurons reduces appetite and body weight in mice (51). CCK<sup>NTS</sup> neurons provide efferent projections to the PVH, where they contact CCK-responsive MC4R neurons. CCK<sup>NTS</sup> neurons also innervate the ARC, although to a lesser extent than the PVH (51). Peripheral CCK, however, released into the circulation on fat ingestion (52), is able to rapidly inhibit AgRP neurons (53). This evidence indicates a key role for CCK as a short-term satiety signal.

The adipokine leptin potently inhibits AgRP neuronal activity (54, 55). Leptin activates the signal transducer and activator of transcription 3 (STAT3), and mice expressing a constitutively active version of STAT3 in AgRP neurons display a lean phenotype and reduced diet-induced obesity (56). Interestingly, using a Cre-loxP approach to selectively delete the leptin receptor (LEPR) on AgRP neurons leads to only a mild increase in body weight and adiposity (57). However, more recently it was shown that employing CRISPR/Cas9-mediated genetic inactivation of the LEPR on AgRP neurons of adult mice resulted in severe obesity and deregulation of glucose metabolism (58). This apparent discrepancy in the phenotype

of mice lacking the *Lepr* in AgRP neurons throughout development and with inducible inactivation of the same gene in adult mice highlights the importance of carefully interpreting findings from gene inactivation studies. Furthermore, this indicates a remarkable degree of plasticity in feeding circuits, if genes or cell types are inactivated at different periods of development. Nevertheless, these data indicate a role for leptin in the regulation of a central anabolic drive via control of AgRP neuron activity.

In contrast to the conceptual framework of homeostasis, which is limited to an invariant set point, the concept of allostasis provides an extended framework for the regulation of AgRP neurons specifically and feeding regulatory neurocircuits in general that allows for control of adaptive physiological and behavioral responses. Here, AgRP neurons sense physiological changes to adapt to future metabolic demands. Specifically, new developments in molecular systems neuroscience, which have enabled the monitoring of neuronal activity of molecularly defined neurons such as AgRP neurons via Ca<sup>2+</sup> imaging in freely behaving mice, have revealed that these neurons transiently adapt their firing properties to sensory food perception as the presentation of food cues produced a rapid inactivation of AgRP neurons (59). Interestingly, prolonged and continuous HFD feeding attenuates the response of AgRP neurons (60), although the exact mechanisms of diet-induced alterations in AgRP neuron regulation remain only partly defined.

In addition to the rapid, food-anticipatory control of AgRP neuron activity, recent elegant work revealed specific vagal afferent types, whose input is relayed via the brainstem, ultimately control AgRP neuron activity in response to intestinal chemosensation and mechanosensation on an intermediate time scale (61). Here, AgRP neurons are rapidly inhibited by activation of mechanoreceptors of stomach-innervating glucagon-like peptide-1 receptor (GLP1-R) neurons and intestine-innervating oxytocin receptor (OXTR) neurons, but this inhibition occurred on different time scales. Activation of GLP1-R neurons leads to a rapid but transient inhibition of AgRP neurons, whereas the activation of OXTR neurons results in a rapid but sustained inhibition. Importantly, while artificial optogenetic activation of these vagal afferents potently suppresses feeding, the necessity of this regulation in the control of day-to-day feeding and its potential deregulation during disease development still awaits further investigation. In fact, a recent study indicated that silencing of GLP1-R-expressing vagal afferent neurons does not alter refeeding responses yet modulates the acute anorexigenic effect of lithium chloride and CCK (62). Thus, the modulatory effect of anticipatory, vagal afferent, and homeostatic feedback regulation not only appears to differ in timing and scale



**Figure 2.** Signal dependent regulation of melanocortin neurons. Agouti-related peptide (AgRP) neurons transiently adapt their firing properties to sensory food perception as the presentation of food cues produced a rapid inactivation of AgRP neurons (59). Sensory food perception activates pro-opiomelanocortin (POMC) neurons and this activation is sufficient to prime metabolic liver adaptation to the postprandial state (63). Furthermore, these neurons receive gut-innervation dependent signals from chemoreceptors and mechanoreceptors on an intermediate time scale and are subject to long-term regulation through homeostatic hormonal regulation. Furthermore, POMC neurons regulate a wide array of different physiological functions, including feeding behavior and energy expenditure, hepatic glucose production, regulation of blood glucose levels, and blood pressure. Similarly, AgRP neurons are involved in feeding behavior and energy expenditure, substrate use, regulation of glucose metabolism, and inflammatory pain.

but also appears to exhibit a remarkable degree of signal specificity.

Collectively, extending the classical view on chronic homeostatic hormonal feedback regulation of AgRP neurons, these recent findings highlight the different temporal dynamics of AgRP neuron regulation, many aspects of which still deserve further detailed investigation (Fig. 2).

### Pro-Opiomelanocortin-expressing Neurons

POMC neurons are located in the ARC and the NTS, and activation of POMC neurons selectively inhibits food intake and body weight gain (24). These neurons are activated by energy surplus and inhibit food intake after prolonged periods of feeding. They integrate long-term adiposity signals from the hypothalamus and short-term satiety signals from the brainstem and release the melanocortins  $\alpha$ - and  $\beta$ -melanocyte-stimulating hormones to activate MC3R and MC4R, thereby reducing food intake and increasing energy expenditure (64-66).

Interestingly, cannabinoids stimulate a switch from  $\alpha$ -melanocyte-stimulating hormone to  $\beta$ -endorphin release from POMC neurons and subsequently increase food intake (67), which argues against a strictly anorexigenic role of POMC neurons.

In addition, POMC neurons are glucose-excited neurons and play a role in the physiological control of systemic

glucose homeostasis as exemplified by chemogenetic inhibition of POMC neurons decreasing glucose levels in normoglycemic animals (68). However, a more recent study revealed that selective activation of liver-innervating POMC neurons increases glucose concentrations (69). Thus, the role of POMC neurons in control of glucose homeostasis appears to be more complex and deserves future detailed studies. However, while functional heterogeneity of distinct POMC neuron populations may account for some of the observed differences between the aforementioned studies, caution should also be paid to the fact that many of the analyses depend on the use of POMC Cre transgenic mice, which express Cre throughout development. As elegantly revealed by the Zeltser laboratory, a substantial proportion of neurons developmentally express POMC, yet later acquire the phenotype of functionally antagonistic AgRP neurons (70). Therefore, use of these constitutive Cre-transgenic mice will mark or delete genes in a subset of functionally antagonistic AgRP neurons, making conclusions of their function selectively in POMC difficult. Clearly, the use of inducible Cre transgenic mice, which circumvent the developmental time window, will help to solve this problem (71).

Interestingly, glucose sensing in POMC neurons is impaired after prolonged feeding of an HFD (72). Furthermore, HFD feeding leads to altered mitochondrial dynamics and mitochondria-endoplasmic reticulum (ER) interactions and

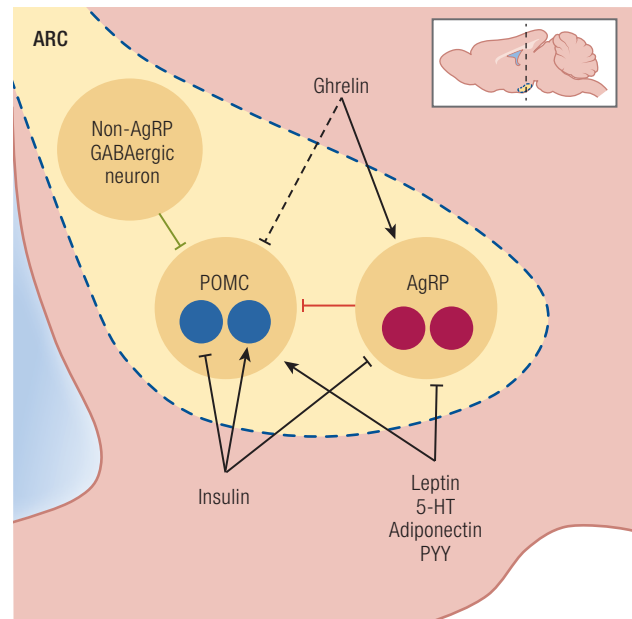
inhibition of POMC neuron firing due to impaired  $\text{Ca}^{2+}$  handling (73-75). Therefore, obesity has profound effects on the signaling capabilities of POMC neurons, which indicates that increased calorie uptake during obesity is, at least in part, mediated by the deregulation of homeostatic POMC neuronal activity.

Different populations of POMC neurons are either inhibited or activated by insulin, which is regulated by the phosphatase TCPTP (T-cell protein tyrosine phosphatase), which is degraded after feeding and increases during fasting (Fig. 3) (76). Activation of IRs on a subset of POMC neurons on the other hand increases the firing of these neurons (77). POMC neuronal activity is suppressed indirectly by ghrelin, most likely via inhibitory GABAergic inputs from activated AgRP/NPY neurons (78). These data are consistent with the emerging concept of molecular and functional heterogeneity of POMC neurons, which clearly represents an important field for future studies (discussed later).

Another hormonal feedback signal, peptide YY (PYY) is released from intestinal L cells and suppresses food intake in humans and rodents (79). Postprandially,  $\text{PYY}_{3-36}$  is the predominant form of PYY in circulation (80).  $\text{PYY}_{3-36}$  inhibits food intake via the activation of POMC neurons (81-83). Peripheral  $\text{PYY}_{3-36}$  may transmit satiety signals to the brain also in part via the vagal afferent pathway since the food intake-reducing effect of PYY is blocked by vagal ligation (84).

Recently, serotonin signaling on POMC neurons has become the focus of several studies. Serotonin (5-hydroxytryptamine, 5-HT) is produced within the CNS, regulates appetite, and increases energy expenditure. In the periphery, circulating serotonin is primarily synthesized by enteroendocrine cells (50, 85). POMC neurons are stimulated and AgRP/NPY neurons are inhibited by serotonin (86-88). Mice with a global deletion of the serotonin receptor 5-hydroxytryptamine receptor 2C (5-HT<sub>2C</sub>R) developed hyperphagia and obesity but on selective reexpression of the 5-HT<sub>2C</sub>R on POMC neurons feeding and body weight normalized (89). In light of these findings, selective serotonin receptor agonists have entered clinical trials for weight reduction (90, 91). However, in early 2020 the 5-HT<sub>2C</sub>R agonist lorcaserin was taken off the US market because of a signal of increased cancer risk (92).

Furthermore, the adipose tissue-derived hormone leptin plays a critical role in whole-body energy homeostasis. Leptin is released by the adipose tissue proportional to fat mass and acts on its cognate receptors (LEPRs) expressed by multiple neuronal groups of the brain (94). Leptin gradually inhibits AgRP neurons and activates POMC neurons gradually on a time scale of hours, while rapid activity changes of these neurons are mediated by sensory food



**Figure 3.** Regulation of arcuate nucleus neurons by circulating hormonal cues. Neurons in the arcuate nucleus of the hypothalamus (ARC) receive hormonal cues and integrate this information to produce adequate metabolic responses. Circles indicate neuronal subpopulations. Ghrelin increases the firing activity of Agouti-related peptide (AgRP)/neuropeptide Y (NPY) neurons through growth hormone secretagogue receptor signaling and inhibits firing activity of pro-opiomelanocortin (POMC) neurons through ghrelin-induced increases in the frequency of GABAergic inhibitory postsynaptic currents of POMC neurons (93). The adipokine leptin potently inhibits AgRP neuronal activity and activates POMC neurons gradually on a timescale of hours. Insulin either activates or inhibits feeding via POMC inhibition or activation, dependent on the regulation of insulin receptor signaling by the phosphatase TCPTP (T-cell protein tyrosine phosphatase) (76).

perception, serotonin, CCK, and PYY (53). Furthermore, leptin acts directly on presynaptic GABAergic neurons and reduces inhibitory tone to postsynaptic POMC neurons (95). LEPR deletion from POMC neurons throughout development leads to the development of mild obesity and hyperleptinemia (96). However, tamoxifen-induced deletion of the LEPR from POMC neurons in adult mice leads to unaltered body weight, food intake, and energy expenditure (97). These animals showed an impaired reduction of leptin levels during fasting, indicating a role of LEPR-expressing POMC neurons in response to fasting via suppression of leptin levels. Mice in which the endogenous LEPR expression was prevented by a *LoxP*-flanked transcription blocker showed severe obesity. Reactivation of LEPR only in POMC neurons using Cre recombinase leads to a modest reduction in body weight but normalized glucose and glucagon levels (98). Interestingly, mice with total hypothalamic POMC deficiency developed severe obesity, whereas mice with restricted POMC expression to hypothalamic neurons expressing the LEPR displayed normal body weight and food consumption (99). Thus, these

studies argue that in the adult organism the predominant food intake suppressing actions of leptin appear to be mediated through LEPRs expressed in neurons other than POMC cells.

Finally, adiponectin, a hormone produced by adipocytes, improves insulin sensitivity. Adiponectin enters the cerebrospinal fluid from the circulation and enhances adenosine 5'-monophosphate-activated protein kinase activity in the ARC via its receptor adiponectin receptor 1 to stimulate food intake (100). Within the ARC, adiponectin exerts glucose- or energy state-dependent opposing effects on POMC neuronal activity and feeding (101).

In addition to the well-defined chronic homeostatic regulation of these cells, the presentation of food cues led to immediate activation of POMC neurons, indicating a role for POMC neurons in anticipation of satiety (59). In line with the notion that POMC neurons regulate not only feeding, but also systemic metabolic homeostasis, POMC neurons sense the impact of food consumption and regulate the adaptation of the liver to a postprandial state. Sensory food perception activates POMC neurons and this activation is sufficient to prime metabolic liver adaptation to the postprandial state through a melanocortin-dependent control of liver sympathetic nerve activity to promote the mammalian target of rapamycin-Xbp1 axis, preparing the organism for the ingestion of food (63). This concept may well extend to the postprandial regulation of adaptive physiological responses in other tissues, a subject clearly deserving of future studies. Moreover, whether the impaired activation of POMC neurons during food anticipation contributes to the multifaceted phenotypes of the metabolic syndrome remains to be investigated.

### Molecular Heterogeneity of Pro-Opiomelanocortin and Agouti-related Peptide Neurons

Recent studies have unraveled the substantial molecular and functional heterogeneity of these classical feeding regulatory melanocortin neurons. Previous studies found that POMC neurons can be classified by the expression of the neurotransmitters glutamate or GABA (102, 103). Furthermore, leptin and 5-HT<sub>2C</sub>R receptors are expressed on distinct subpopulations of POMC neurons (104). Using single-cell RNA sequencing, Lam et al (105) identified 25% of POMC neurons that coexpressed AgRP, indicating shared developmental origins. They also revealed a high degree of heterogeneity of POMC neurons in their data. This finding is in line with a study by Campbell and colleagues (106) that identified several distinct subsets of POMC and AgRP neurons. Unbiased clustering analysis revealed 3 main clusters of POMC neurons (n14.Ttr, n15.Anxa2, and

N21.Glipr) and 2 main clusters of AgRP neurons (n12.SST and n13.Gm8773).

The obvious heterogeneity of these neurons requires the development of novel genetic tools to dissect the relevance of these neuronal populations in feeding regulation. Combining Dre/roX recombinaSe technology with a Cre/loxP recombination allows us to specifically express transgenes in a defined subset of neurons. Employing these tools, our group was able to demonstrate the principal feasibility of these technologies to selectively target Lepr- and GLP-1R-expressing POMC neurons (107). In fact, using different mouse lines allowed us to visualize these distinct neuronal populations, performing specific ribosomal profiling of these cell types, as well as the ability to selectively modulate the activity of molecularly defined POMC subpopulations, revealing that both Lepr- and GLP-1R-expressing POMC neurons represent largely nonoverlapping cell types with differential anatomical localization in the ARC, distinct expression profiles for neuropeptides and neuropeptide receptors, as well as differentially regulated feeding on acute chemogenetic activation (107). These studies clearly highlight the complexity of metabolism regulatory neurocircuits even within previously considered homogenous cell types such as POMC neurons. Further deciphering the specific functions and neurocircuit integration of distinct subclusters of these cells may pave the way to more specific pharmacological modification of distinct metabolic effector pathways controlled by these cell types (for review, see Quarta et al) (108).

### Effector Pathways of Melanocortin Neurons in the Arcuate Nucleus of the Hypothalamus

#### The paraventricular nucleus of the hypothalamus

The PVH incorporates neuroendocrine information from other brain regions with autonomic nervous system functions, including afferent inputs as well as autonomic outputs. AgRP and POMC neurons provide projections to PVH neurons that express MC4R, which is highly abundant in the PVH (109). Selective reexpression of MC4R expression in MC4R null mice showed that the MC4R expressed in the PVH and amygdala control food intake, whereas MC4R expressed in other brain sites are involved in controlling energy expenditure (19). In line with this finding, chemogenetic activation of PVH MC4R neurons, mimicking stimulation by endogenous POMC ligands, significantly reduced food intake without inducing an aversive response (20), indicating a role of these neurons as mediators of food intake inhibition under physiological conditions. Similarly, surgical disruption of ARC → PVH projections leads to an increase in body weight (110). Furthermore, stimulation of AgRP axonal projections to



the PVH is sufficient to evoke feeding similar to AgRP somatic activation (35). Interestingly, brief exposure of adult mice to an HFD significantly decreases AgRP-immunoreactive neuronal projections to the PVH (111). Conversely, glutamatergic neurons from the PVH, namely thyrotropin-releasing hormone and pituitary adenylate cyclase-activating polypeptide-expressing neurons, provide axonal projections to the ARC and provide excitatory input to AgRP neurons (112). Thus, further delineating the exact nature of PVH-effector pathways may provide novel targets to bypass leptin resistance of melanocortin neurons in the ARC as observed in obesity (113-116).

### The lateral hypothalamus

The LH integrates reward and appetitive behavior to regulate motivated feeding behavior and positive enforcement (117, 118). Hypocretin/Orexin neurons in the LH are activated by fasting and low glucose levels and are rapidly inactivated on food intake (119, 120). Their appetite-enhancing effects might be mediated via suppressing hypothalamic POMC neuronal activity (121). Another neuronal population in the LH, neurons expressing MCH, are involved in the regulation of feeding. Activation of MCH receptors or intracerebroventricular injection of an MCH-1 receptor agonist increases food intake and facilitates body weight (122). Furthermore, overexpression of MCH in the LH leads to high plasma insulin levels and a blunted response to insulin (123). The role of MCH neurons in feeding is mediated via inhibition of POMC neuronal activity, and the SIRT1/forkhead box protein O1 mediates MCH control on food intake and glucose metabolism (124).

### The dorsomedial nucleus of the hypothalamus

The dorsomedial hypothalamus (DMH) contains MC4R-expressing neurons (109) and plays an important role in regulating adaptive thermogenesis. The knockdown of NPY in the DMH promotes brown adipose tissue activity and increases body energy expenditure (125). Furthermore, DMH neurons fine-tune feeding regulatory neurons in the ARC. LEPR-expressing GABAergic neurons in the DMH provide inhibitory input to AgRP neurons (126). In addition, DMH → POMC GABA circuitry conveys inhibitory neuromodulation onto POMC cells (127).

### Bed nucleus of the stria terminalis

The BNST forms a continuum with the amygdala and is characterized as a center for mediating behavioral responses to anxiety and stressor exposure (128). The role of BNST neurons in the regulation of food intake is relatively unexplored. However, some studies indicate that manipulations of the BNST neurocircuitry have profound effects on food intake and reward. Food consumption was

shown to activate BNST neurons (129) and BNST lesions result in hyperphagia and obesity, indicating that reduced BNST output promotes feeding (130). Indeed, inhibition of GABAergic BNST neurons diminishes food intake (131). In addition, activation of BNST-projecting AgRP neurons was sufficient to induce feeding (35) and to induce insulin resistance on AgRP neuron activation, and conversely projections of prepronociceptin (PNOC)-expressing neurons in the BNST regulate feeding via regulation of AgRP neurons (132).

### Parabrachial nucleus

Neurons in the PBN are associated with appetite control and relay visceral sensory information that inhibits feeding (133).

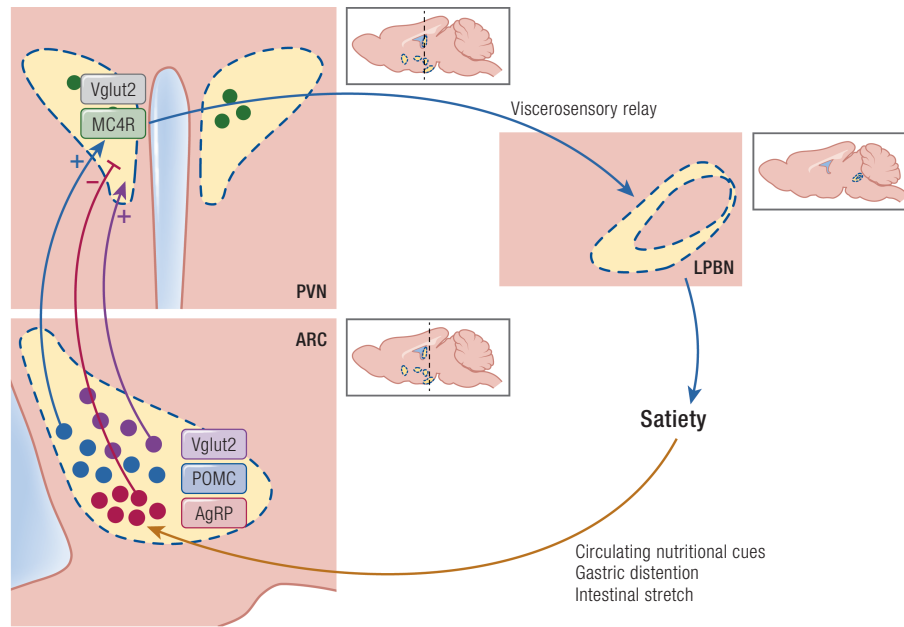
PVH<sup>MC4R</sup> neurons are a critical satiety-promoting population and act as a second-order node in the regulation of feeding. PVH<sup>MC4R</sup> neurons project to the lateral parabrachial nucleus (LPBN) and optogenetic stimulation of PVH<sup>MC4R</sup> → LPBN projections promotes satiety, establishing the LPBN as a possible third-order node in feeding regulation (20) (Fig. 4).

Previous research established an anorexigenic circuit arising from calcitonin gene-related peptide (CGRP) neurons in the LPBN. Optogenetic and chemogenetic activation of LPBN<sup>CGRP</sup> neurons decreases appetite (136). Conversely, inhibition of CGRP neurons increases the size of individual feeding bouts without increasing total food consumption, indicating a role of LPBN<sup>CGRP</sup> neurons for meal termination (133). In addition, optogenetic activation of AgRP fibers in the PBN delays satiation by inhibiting CGRP-expressing neurons (137). However, PVH<sup>MC4R</sup> neurons do not engage LPBN<sup>CGRP</sup> neurons (20), but glutamatergic LPBN<sup>GLUT2</sup> neurons receive input from PVH<sup>MC4R</sup> neurons (138).

### Other feeding-regulatory arcuate nucleus of the hypothalamus neurons

Given the pivotal role of these neurons in the regulation of energy homeostasis, it is remarkable that AgRP neuronal activity is not required for obesity development and, when palatable food is provided, AgRP neurons are dispensable for an appropriate feeding response (31, 139). In addition, prolonged HFD feeding attenuates the response of AgRP to feeding-related stimuli (60). This strongly indicates a role for ARC non-AgRP GABAergic neurons in chronic control of appetite and body weight regulation.

There are several lines of evidence indicating that GABA-expressing non-AgRP neurons in the ARC regulate obesity in mice. Tyrosine hydroxylase neurons in the ARC increase appetite via dopamine-mediated excitatory action on AgRP and inhibition of POMC neurons.



**Figure 4.** Neuronal regulation of feeding. Activation of agouti-related peptide (AgRP) neurons potentially promotes feeding, whereas activation of proopiomelanocortin (POMC) neurons decrease feeding over longer time scales. AgRP and POMC neurons send projections to the paraventricular nucleus of the hypothalamus (PVH) neurons that express melanocortin 4 receptor (MC4R), which is highly abundant in the PVH (109, 134). A fast-acting satiety mechanism is conveyed by glutamate-releasing oxytocin-receptor expressing (Vglut2/OxtR) neurons in the arcuate nucleus of the hypothalamus (ARC). Excitatory Vglut2/OxtR projections synaptically converge with GABAergic AgRP projections on PVH<sup>MC4R</sup> neurons and rapidly cause satiety (135). PVH<sup>MC4R</sup> neurons are a critical satiety-promoting neuronal population and act as a second-order node in the regulation of feeding. In the PVH, MC4R-expressing neurons release glutamate and excite downstream neuronal targets in the lateral parabrachial nucleus (LPBN), establishing the LPBN as possible a third-order node in feeding regulation (20).

Furthermore, these neurons inhibit PVH neurons by dopamine and GABA corelease (140). Another recently identified GABAergic cell type in the ARC are prepronociceptin (PNOC)-expressing neurons. These neurons are activated on short-term HFD feeding. Optogenetic stimulation of ARC PNOC neurons increased feeding via direct monosynaptic GABAergic inhibition of POMC neurons. In addition, stimulation of PNOC ARC → BNST axonal projections contributes to the feeding stimulatory effect. Selective ablation of ARC PNOC neurons reduces HFD intake and protects from obesity (141). Furthermore, PNOC neurons in the central amygdala promote palatable food consumption and reward (142). PNOC neurons in the BNST inhibit feeding via axonal projections to AgRP neurons to regulate food intake (132), indicating a significant role of PNOC-expressing circuitries in feeding regulation, palatable food consumption, and the development of obesity. This finding is in line with a recent study showing that the chronic activation of hypothalamic ARC non-AgRP GABAergic neurons leads to obesity, whereas chronic inhibition of ARC GABAergic neurons reduces (aging-related) weight gain (134).

Furthermore, glutamate-releasing neurons in the ARC that coexpress OXTR (ARC<sup>Vglut2/OxtR</sup>) rapidly cause satiety on chemogenetic or optogenetic activation by changing the plasticity of excitatory input onto PVH<sup>MC4R</sup> neurons (135).

Thus, recent studies have revealed important roles for nonmelanocortin neurons in ARC-dependent control of feeding, particularly during obesity development. Further characterizing these novel pathways, defining their cell-autonomous regulators as well as upstream and downstream effector neurocircuits, may set the groundwork for the development of novel strategies to fight the obesity epidemic.

#### Nonneuronal feeding-regulatory cell types

Recent evidence shows that nonneuronal cell types in the ARC are involved in the regulation of energy balance. These cells are integrated into the network of neuronal cells in the ARC. Astrocytes, a glial cell type that receives more and more attention, contribute to the maintenance of homeostasis via control over the blood-brain barrier (neuro-glio-vascular unit and glial-vascular interface), the regulation of local and systemic blood flow, metabolic support and glycogen synthesis and storage (143, 144).

Optogenetic and chemogenetic activation of ARC astrocytes was shown to reduce the firing of AgRP neurons via modulation of extracellular levels of adenosine, resulting in reduced food intake (145, 146). Astrocytes moreover play an important role in control of glucose uptake into the brain and the regulation of feeding as astrocyte-specific deletion of the IR reduces glucose-induced activation of

POMC neurons (147). Furthermore, the deletion of the LEPR on astrocytes led to a reduction of leptin-regulated feeding (148). Here, astrocytes appear to integrate an increasing number of metabolic cues, as also deletion of the GLP1-R affects fuel use of astrocytes, alters neuronal responses, and changes brain glucose uptake (149). Thus, defining, the fine-tuned interaction of neurons and glia cells in feeding circuits promises to define new regulatory mechanisms and potentially targets for intervention with central metabolism-regulatory neurocircuits.

Finally, ingestion of an HFD causes the release of inflammatory mediators from nonneuronal cell types, such as microglia, which contributes to long-lasting impaired metabolic control of hypothalamic neurons (150, 151) (for review, see Jais and Brüning) (152).

## Conclusion and Outlook

The hypothalamus integrates neuroendocrine and autonomic systems and coordinates metabolic responses across multiple tissues. These key feeding regulatory neurocircuitries overlap with brain centers involved in food-related reward, such as the amygdala, striatum, and ventral tegmental area and helps translate the homeostatic regulation of feeding into motivated behavior (for reviews, see Rossi and Stuber, and Berthoud et al) (153, 154). Obesity alters the responsiveness to circulating feedback signals and the neural plasticity of feeding-regulatory neurocircuits, which leads to persistent changes in energy balance and body weight (155).

While AgRP- and POMC-expressing neurons in the ARC have been identified as key regulators of not only feeding control, but also of integrative physiology during adaptation to the current energy status of the organism, recent experiments have revealed new levels of complexity in this regulation. These findings include the dynamic regulation of these neurocircuits in response to sensory food perception, nutrient-induced regulation via vagal afferents and ultimately via long-term homeostatic feedback hormonal signals. Defining the regulatory mechanism of this stimulus- and meal timing-dependent regulation of not only feeding responses but also of glucose metabolism will broaden our understanding about the complex regulation of metabolism through the CNS. Moreover, the recent discovery of the heterogeneous nature of metabolism-regulatory neurons, previously considered homogenous cell types, opens an entirely new research area to define the specific physiological responses governed by molecularly defined neuronal subtypes, and ultimately carries the promise for more targeted, specific pharmacological intervention.

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