Role of astrocytes, microglia and tanycytes in brain control of systemic metabolism

[1]García-Cáceres C., [2]Balland E., [3]Prevot V., [4]Luquet S., [5]Woods S.C., [6]Koch M., [7]Horvath T.L., [8]Yi CX., [9]Chowen J.A., [10,11]Verkhratsky A., [12]Araque A., [6]Bechmann I. & [1]Tschöp M.H.[*]

[1]Helmholtz Diabetes Center, Munich, Technische Universität München & German Center for Diabetes Research.

[2]Department of Physiology, Biomedicine Discovery Institute, Monash University, Clayton, VIC 3800, Australia

[3]Inserm, Laboratory of Development and Plasticity of the Neuroendocrine Brain, Centre de recherche Jean-Pierre Aubert, UMR_S1172, bâtiment Biserte, 1, place de Verdun 59045 Lille Cedex, France - Université de Lille, Faculté de Médecine, Lille, France.

[4]Univ Paris Diderot, Sorbonne Paris Cité, CNRS UMR 8251, F-75205, Paris, France.

[5]Department of Psychiatry and Behavioral Neuroscience, University of Cincinnati, 2170 Galbraith Avenue, Cincinnati, OH 45237, USA.

[6]Institute of Anatomy, Leipzig University, Leipzig, Germany.

[7]Department of Comparative Medicine, Yale University School of Medicine, New Haven, CT, 06510, USA.

[8]Department of Endocrinology and Metabolism, Academic Medical Center Amsterdam, 1105 Amsterdam, The Netherlands.

[9]Department of Endocrinology, Hospital Infantil Universitario Niño Jesús, Instituto de Investigación la Princesa; IMDEA Food Institute, CEI UAM + CSIC; CIBEROBN, Instituto Carlos III Madrid, Madrid, Spain.

[10]Faculty of Biology, Medicine and Health, The University of Manchester, Manchester, UK.

[11]Achucarro Center for Neuroscience, IKERBASQUE, Basque Foundation for Science, 48011 Bilbao, Spain.

[12]Department of Neuroscience, University of Minnesota, Minneapolis, MN, USA.

[*]Author for correspondence (tschoep@helmholtz-muenchen.de)

Prof. Dr. Matthias H. Tschöp, M.D.

Institute for Diabetes and Obesity

Helmholtz Center Munich

Parkring 13

85748 Garching-Hochbrück, Germany.

Abstract

Astrocytes, microglia and tanycytes play active roles in the regulation of hypothalamic feeding circuits. These non-neuronal cells are crucial in determining the functional interactions of specific neuronal subpopulations involved in the control of metabolism. Recent advances in biology, optics, genetics and pharmacology resulted in the emergence of novel and highly sophisticated approaches for studying hypothalamic neuronal-glial networks. Here we summarize the progress in the field and argue that glial-neuronal interactions provide a core hub integrating food-related cues, interoceptive signals and internal states to adapt a complex set of physiological responses operating on different time scales to finely tune behavior and metabolism according to metabolic status. This expanding knowledge helps to redefine our understanding of the physiology of food intake and energy metabolism.

Introduction

The brain of mammals contains billions of cells with extensive molecular, morphological and functional diversity. These cells are precisely interconnected throughout the Central Nervous System (CNS) to form intricate and dynamic circuits, each performing specific functions. Over last decades, specific hypothalamic neuronal networks have emerged as key orchestrators of systemic metabolism, food intake and body weight. However, restricting our scope to neuronal circuitry may have contributed to poor success in discovering and developing more effective and safe drugs for obesity. Astrocytes, tanycytes and microglia, as well as neuronal-glial interactions, have recently proven to be highly relevant for the control of systemic metabolism and should lead to improved pharmacological strategies to prevent and treat metabolic diseases. Here we review these emerging insights to argue that achieving effective regulation of metabolic homeostasis will require understanding the functional heterogeneity and interactions of all hypothalamic cells.

Focus on the hypothalamus

Identification of the hypothalamus as a center of metabolic homeostasis arose from observations that hypothalamic damage inflicted by either tumors [1] or by lesions to specific hypothalamic regions, including the ventromedial (VMH), dorsomedial (DMH) and paraventricular (PVN) nuclei [2-4], elicited voracious hunger (hyperphagia) and obesity. Thus, these early studies of hypothalamic dysfunction suggested a critical role of the hypothalamus in regulating metabolism. When lesions to the lateral hypothalamus (LH) were found to reduce food intake (hypophagia) [5], it became clear that there is intra-regional variation of hypothalamic regulation of food-seeking behavior and body weight.

The subsequent discoveries that circulating metabolic hormones such as insulin [6], leptin [7] and ghrelin [8] act at the hypothalamus, together with the implementation of genetically-engineered rodent models of obesity and diabetes [9,10] and cell-specific ablation studies [11], promoted the identification of specific hormone-sensitive hypothalamic cell populations and facilitated the deciphering of the functional properties of diverse hypothalamic feeding circuits. Understanding the functional and cellular heterogeneity of distinct neuronal populations forming these circuits, as well as the intricate networks

interconnecting them, are essential for deciphering how the brain controls energy metabolism (see review: [12]).

Hypothalamic neuronal networks

Arcuate nucleus

The arcuate nucleus (ARC) in the ventral floor of the mediobasal hypothalamus (MBH) abuts the median eminence (ME), one of the brain's circumventricular organs (CVOs). CVOs are midline areas characterized by the presence of fenestrated capillaries allowing the passive diffusion of blood-borne molecules. This characteristic allows a wider accessibility of nutrient and energy-related signals between the blood and the extracellular fluid bathing adjacent neuronal networks of the ARC. ARC neurons that extend dendrites into the ME [13] are thus capable of directly transforming metabolic cues into neuronal signals, and they are considered the "first-order" neurons which receive and integrate metabolic signals. Axons of these neurons project widely onto diverse "second-order" neurons which have been extensively studied for their involvement in the regulation of energy intake and body weight. Important findings include the discovery of the orexigenic properties of neuropeptide Y (NPY) [14] and Agouti-related peptide (AgRP) [15], and the anorexigenic properties of α-melanocyte-stimulating hormone (α-MSH), the post-translational product of proopiomelanocortin (POMC) [16].

NPY/AgRP neurons – linking metabolism with behavior

The discovery of the co-expression of two potent orexigenic peptides, NPY and AgRP, within the same ARC neurons provided compelling evidence that these cells are the long-sought hunger neurons. New methodologies, including cell-type specific ablation and acute modulation of neuronal activity combined with tracing methods, have provided detailed views of how these neurons influence both feeding and non-feeding-related behaviors. Ablation of AgRP neurons leads to starvation and death [11,17], while optogenetic or pharmacogenetic activation or inhibition [18] of AgRP neurons *in vivo* positively or negatively affects feeding, respectively. Although AgRP antagonizes melanocortin (MC) receptors, this may not be its underlying mechanism since acute stimulation or ablation of AgRP rapidly affects feeding during tonic inhibition of the MC system, implying that AgRP neurons act independently of acute MC signaling [19]. When food is available, activating the soma of AgRP neurons increases food consumption, whereas the same stimulation in the absence of food increases stereotypic and compulsive behaviors [20]. Optogenetic or pharmacogenetic excitation of AgRP neurons – as a proxy for what occurs in food deprivation – revealed that, depending on the experimental context and food availability, activation of these cells can promote adaptive responses facilitating escape from the state of deprivation. Then, when food is discovered, this behavior can immediately revert, indicating that AgRP neurons have the ability to rapidly respond to food-related cues rather than to calories *per se* [21]. Indeed, *in vivo* calcium imaging of AgRP neurons in freely moving animals has demonstrated their rapidly reductions in activity upon detection of food cues, even before substantial calories are consumed [22,23].

Multiple brain regions receive direct inputs from ARC AgRP neurons (Figure 1A), and direct stimulation of these individual projections revealed a parallel and redundant signaling network by which they promote feeding. Direct stimulation of the subset of AgRP axons that specifically project to the PVN ($ARC^{AgRP} \rightarrow PVN$), to the anterior division of the bed nucleus of the stria terminalis (aBNST), to the paraventricular thalamus (PVT), or to the LH [24], elicits acute feeding. Acute optogenetic activation and tracing studies of AgRP neuronal projections revealed that different subsets of AgRP neurons control insulin sensitivity, glucose metabolism and feeding through distinct and overlapping projections [25]. ARC^{AgRP} to LH projections promote feeding and insulin resistance through modulation of brown adipose tissue (BAT) metabolism, and ARC^{AgRP} to $aBNST^{vl/dm}$ projections coherently but independently regulate feeding and stimulate expression of muscle-related genes in BAT in addition to glucose uptake [25]. In contrast, acute stimulation of AgRP projections to the central nucleus of the amygdala (CeA), the periaqueductal gray area (PAG), or the parabrachial nucleus (PBN) do not trigger feeding [24]. Accordingly, genetic or pharmacologic inhibition of AgRP fibers projecting to the PBN prevents the starvation that results from ablation of all AgRP neurons [26]. Thus, AgRP neurons influence multiple aspects of feeding behavior via diverse neuronal circuits throughout the brain. AgRP neurons also regulate peripheral activity through modulation of autonomic output. Ablation or hypomorphic AgRP activity highlight additional roles of AgRP neurons unrelated to feeding, including regulation of the balance between carbohydrate and lipid utilization [27], adaptive immune responses and T cell maturation [28], and the norepinephrine-dependent control of bone mass [29].

POMC neurons: center of body weight regulation

Reciprocal activity of ARC POMC and AgRP neurons is fundamental for hypothalamus-driven control of whole-body energy balance. Alternating firing of AgRP and POMC neurons is achieved by cell type-specific effects of metabolic hormones, circulating nutrients and prandial state-dependent synaptic inputs onto both cell types [30]. During a meal, POMC neurons become activated, leading to gradual onset of satiation and increased energy expenditure [31]. Independent of food consumption *per se*, POMC neurons rapidly adapt their activity in response to external information about food availability and food composition [22,23]. Thus, the sensory detection of food in overnight-fasted mice leads to a paradoxical activation of satiation-promoting POMC neurons without actual food intake [23]. Activation of POMC neurons results in the release of several POMC-derived peptides including α-MSH that gradually promote the onset of satiation and increased energy expenditure via activation of MC3/4Rs in the PVN and the nucleus of the solitary tract (NTS) in the brainstem [32]. ARC POMC neurons also secrete β-endorphin, which, when acutely released into the PVN, triggers feeding in response to cannabinoids in sated mice [32]. Chemogenetic and pharmacological studies indicate that POMC neurons drive acute cannabinoid-induced feeding through cannabinoid-mediated retrograde inhibition of presynaptic GABA release onto POMC cells (Figure 1B), and postsynaptic formation of contacts between mitochondria and endoplasmic reticulum (ER) in POMC cells. Leptin induces the formation of reactive oxygen species (ROS) in POMC neurons, and this is required to trigger POMC neuronal activity to increase energy expenditure and promote satiation [33].

POMC neurons project to different brain areas (Figure 1B), with neurons in the rostral ARC projecting mainly to autonomic brainstem regions and neurons in the caudal ARC primarily connecting with other hypothalamic nuclei such as the PVN [34]. The region-specific distribution of ARC POMC neurons also determines their responsiveness to metabolic factors, with those in the medial ARC being predominantly sensitive to glucose, while those in the lateral ARC are more responsive to leptin [35]. Although most POMC cells have functionally active leptin receptors (LepRs), those which do are not more responsive to insulin. One population of POMC cells is insensitive to both leptin and insulin but is activated by serotonin or estrogen [34]. In sum, POMC neurons represent a heterogeneous population that orchestrates fundamental functions in the maintenance of energy homeostasis, including the regulation of feeding behavior and energy expenditure and maintenance of blood glucose levels.

Other key hypothalamic neuronal populations

Numerous neuronal populations in the ARC and other hypothalamic nuclei are required for the physiological control of energy metabolism [36]. One subpopulation of ARC neurons expresses oxytocin receptors and releases glutamate, but does not contain POMC. Rather, it promotes rapid onset of satiation by activating ARC-to-PVN projections [37]. Orexigenic dopaminergic (DA) neurons that reciprocally control the activity of nearby AgRP and POMC cells have recently been characterized in the ARC [38]. Ghrelin triggers increased activity of these ARC DA neurons through affecting AgRP and POMC neurons. In addition to their acute effects on feeding, ARC DA neurons inhibit lactotrophs in the anterior pituitary, preventing lactation [39]. In RIP-Cre transgenic mice, the neurons expressing Cre (RIP-Cre neurons) are located throughout the brain including the ARC but are distinct from POMC and AgRP neurons. RIP-Cre neurons exert GABAergic inhibition onto PVN neurons and mediate increased energy expenditure, but do not decrease feeding associated with leptin [40].

Other hypothalamic nuclei also contain specific neuronal populations that participate in the regulation of energy metabolism. In the LH, hypocretin/orexin-expressing glutamatergic neurons project to several hypothalamic nuclei and other brain areas, promoting arousal and food intake [41,42]. Neighboring LH melanin-concentrating hormone (MCH) neurons are GABAergic and elicit hyperphagia. Finally, GABAergic projections from LH neurons to the ventral tegmental area (VTA) drive vigorous feeding [43]. In contrast to the orexigenic nature of the LH, the VMH primarily promotes reduced food consumption and consists of heterogeneous subtypes of neurons expressing leptin receptors (LepRs), estrogen receptor α and/or receptors for brain-derived neurotrophic factor (BDNF) [44]. Steroidogenic factor-1 (SF-1) is a well-known cell marker that, in the brain, is selectively produced by neurons in the VMH [45]. These neurons regulate thermogenesis and promote leptin's effects on energy expenditure; malfunctioning of SF-1 neurons is related to the onset of obesity [46].

Collectively, the hypothalamus comprises numerous distinctive neuronal populations and controls energy balance in multiple ways utilizing diverse signals. However, the feeding circuits also extend to extra-hypothalamic areas (Box 1) of the CNS and pituitary to integrate metabolic information for rapidly adapting to new situations. Adaptive plasticity of hypothalamic neuronal connectivity (Box 2)

requires the coordinated participation of adjacent glial cells which together with neurons chemically and physically influence fundamental aspects of synaptic function.

Box 1. Connectivity with extra-hypothalamic metabolic control circuits

Considerable peripheral metabolic information reaches the brain through afferent nerves originating from the gastrointestinal tract, including the vagus nerves, that terminate in the NTS, and NTS neurons subsequently project to other brain areas including the hypothalamus [47]. The brainstem has a high level of MC4R expression [48], implying that extra-hypothalamic areas also actively control energy balance via MC signaling. Of note, MC4Rs in autonomic regions of the brainstem regulate energy expenditure and glucose homeostasis, whereas MC4Rs in the hypothalamic PVN control feeding but not energy expenditure [49,50]. Such region-dependent functionality of MC4Rs highlights the divergent character of the MC system in controlling energy homeostasis and the relevance of interconnections between the hypothalamus and extra-hypothalamic regions.

Feeding behavior, in addition to being influenced by homeostatic signals, is affected by emotional, social, experiential and many other factors, any of which can interact with meal timing, size, choice and preference, overriding homeostatic controls. The mesolimbic dopamine pathway originating in the VTA project onto the nucleus accumbens (NAc) and other brain regions including the LH [51]. These dopaminergic pathways potently alter motivated "wanting" for the food reward [52] and are directly impacted by leptin [53], insulin [54] and ghrelin [55]. Mechanisms involved in the activation of DA signaling remain unclear, albeit activated brain areas vary depending on metabolic status.

Past experiences (*e.g.,* learning and memory) greatly influence the control of eating and conditioned-appetitive behaviors [56]. In fact, lesions in the hippocampus promote hyperphagia and adipose deposition [57]. The hippocampus expresses receptors for and can respond to hormonal cues including ghrelin and insulin, contributing to both the homeostatic control of metabolism as well as to hippocampal-dependent learning and memory [58,59]. Recent studies have uncovered a top-down pathway originating from the medial prefrontal cortex, connecting to somatostatin-positive neurons of the lateral septum to the LH, for controlling food seeking behavior and evoking food approaches without affecting food intake [60]. Indeed, the LH rapidly responds to inputs from the lateral septum in the course of food seeking in mice, indicating that cortical- or hippocampal- to hypothalamic connections are required for sensory food detection as well as for food seeking [22,23,61].

Box 2. Synaptic plasticity in the hypothalamic regulation of metabolism

The mammalian brain retains the capacity for structural reorganization and functional adjustments of synaptic transmission throughout life [62]. This ability is known as synaptic plasticity and is fundamental for the organism to adequately adapt behavioral outcomes in response to energy demands, environmental experiences and learning processes. The brain adjusts both cortical and hippocampal synaptic transmission in response to experience and learning, and also modulates the connectivity of brain circuits in the hypothalamus in response to metabolic shifts. Consequently, hypothalamic-driven

control of feeding behavior and energy expenditure are affected by synaptic plasticity throughout life [63]. This involves synaptic input re-arrangements based on dynamic synapse formation and elimination, processes fundamental to achieve adequate postsynaptic responsiveness of AgRP and POMC neurons to distinct transmitters after a shift in metabolic state. Rapid synaptic remodelling of ARC feeding circuits is initiated by leptin, ghrelin and estradiol [30]. Metabolism-dependent rewiring of synaptic input organization has also been observed in the LH, involving fasting or endocannabinoid-mediated switches in the synaptic input organization of orexinergic neurons [64].

Glia

Astrocytes: active members of the circuit

Astrocytes are highly diverse in their morphological appearance, functional properties and distribution, both among and within different brain regions [65,66]. The majority of protoplasmic astrocytes in the CNS do not have the classic textbook stellate morphology; rather, they resemble "sponges" because of their highly elaborated, plastic and dynamic terminal process arborization [67]. Astrocytes sense synaptic activity through the expression of neurotransmitter receptors, transporters and ion channels, enabling them to control the concentrations of ions, neurotransmitters and neurohormones in the extracellular space; astrocytes also supply adjacent neurons with glutamine, the obligatory precursor for glutamate and GABA. Astroglia are secretory cells (the gliocrine system), releasing numerous neuroactive molecules including classical neurotransmitters [66]. The bidirectional communication between astrocytes and neurons was initially embodied in the concept of the tripartite synapse [68], that evolved into the multipartite synaptic cradle that includes both astrocytes and microglia as integral elements of the synaptic formation [66]. Astrocytes modulate neuronal activity and synaptic transmission in several brain areas [69] and support synaptogenesis, synaptic maturation, maintenance and extinction. Astroglial cells are organized into networks where extensive communication occurs through gap-junction channels that mediate intercellular signaling in the form of Ca^{2+} or Na^{+} waves [70], as well as activity-dependent glucose delivery and trafficking of glucose metabolites from capillaries to distal neurons [71] in the CNS, including the hypothalamus [72]. Hypothalamic astrocytes have been reported to play a critical role in regulating nutrient and hormone sensing within the CNS [73,74].

Hypothalamic astrocytes are highly glucose-responsive

Astrocytes play a key role in the transport and sensing of glucose via gap junctions [75], the astroglial glucose transporter (GLUT)-1 [76], and the insulin receptor [73]. Insulin signaling in hypothalamic astrocytes participates in the transport of glucose from the blood into the brain, and these astrocytes regulate the glucose-induced activation of POMC neurons that is critical for reducing food intake and controlling systemic glucose metabolism [73]. The loss of connexin 43 in astrocytes inhibits wake-promoting LH orexin neurons by impairing glucose and lactate trafficking through astrocytic networks [72]. Although the morphology and physiology of hypothalamic astroglia have yet to be fully characterized, specific subtypes have been identified. One subtype that is abundant in the ARC is Gomori-positive (GP) cytoplasmic granules derived from degenerating mitochondria that have a

particularly high affinity for Gomori's chrome alum hematoxylin and toluidine blue stain [77]. The GP astrocytes are mainly found together with tanycytes (see below) in areas of the ARC highly enriched in GLUT-2 protein and that have elevated glucose-dependent oxidative metabolism, features that might influence the functional activity of adjacent neurons [78]. These findings demonstrate that astrocytes differ not only among different brain areas, but also within them, which is also true for the hypothalamus [79,80].

The phenotype and functionality of hypothalamic astrocytes is determined by adjacent neurons and the local microenvironment; thus, astrocytic activity might change in response to the energetic needs/demands of their surrounding extracellular space. Stimulation of the PVN with norepinephrine or glutamate stimulates astroglial ATP release, leading to the enhancement of synaptic efficacy at glutamatergic synapses [81,82]. Astrocytes can act as metabolic sensors regulating food intake by rewiring hypothalamic circuits to ultimately balance energy metabolism via such signals [74,83]. MBH astrocytes alter the firing rate of AgRP neurons for the bidirectional regulation of leptin- and ghrelin- regulated feeding circuits via release of adenosine and activation of adenosine A_1 receptors [83] (Figure 2). In addition MBH astrocytes express functional leptin and insulin receptors. Selective genetic loss of these receptors in adult mice reduces the ability of leptin and glucose to suppress food intake [73,74].

Tanycytes and the blood-brain barrier (BBB)

Tanycytes are specialized, unciliated ependymoglial cells (which belong to astroglia class) that line the floor of the third ventricle in the tuberal region of the hypothalamus near the ARC [84]. Although tanycytes share many common features with astrocytes, they display a unique morphology and distinct functional characteristics [85]. Tanycytes are polarized cells, with cell bodies located in the wall of the third ventricle and elongated processes extending into the parenchyma and contacting the pial surface of the brain. Due to this peculiar morphology and their stem cell properties, tanycytes can be considered as radial glia of the mature brain [84]. In the ME, tanycytes contribute to the regulation of key hypothalamic functions including reproduction and metabolism. They dynamically control neuropeptide secretion into the hypothalamo-pituitary portal circulation, sense and respond to local glucose levels, generate the active form of thyroid hormones and regulate local homeostasis via their ability to control the exchange of molecules such as leptin between the blood and the hypothalamic extracellular fluid [84].

The BBB is critical for maintaining the brain microenvironment as it prevents the direct exposure of the parenchyma and the cerebrospinal fluid (CSF) to the blood with their potentially toxic circulating molecules. The ME-barrier dynamics involve a complex coordination between tanycytes and endothelial cells. Tanycytes possess organized tight-junction complexes at the level of their cell bodies that seal the intercellular space, preventing the free diffusion of blood-borne molecules extravasating from the ME fenestrated capillaries into the CSF (Figure 2) [84]. On their opposite pole, tanycytes contact either the ME microvessel loops in the ventromedial ARC or the BBB vessels proper within the ARC itself [84]. In the ME-ARC region the organization of tight junctions in tanycytes and tanycyte-mediated

endothelial cell fenestration is plastic, and can be reorganized to modulate the direct access of blood-borne metabolic signals such as glucose and ghrelin to nearby ARC neurons and, consequently, regulate the adaptive response to acute nutritional challenges [86,87] (Figure 2). Previous studies suggested that metabolic hormones enter the brain mainly by transport across the vascular endothelium [88]. Recent evidence, however, indicates that peripheral peptides also enter the brain via transcytosis across tanycytes from the ME to the third ventricle where they can circulate through the ventricular system and access the ARC and other distant brain structures lining the ventricles [89]. Blood-borne leptin freely exits the circulation through fenestrated capillaries and is taken up by tanycytes, which are the first cells in the hypothalamus to perceive and respond to this circulating hormone (Figure 2) [89]. Likewise, circulating ghrelin is also transported to the CSF by tanycytes (Figure 2) [90].

Microglia and hypothalamic immunity

Microglia, phagocytic and antigen-presenting cells in the brain, are neuroprotective through the secretion of neurotrophic factors such as BDNF, and are also crucial for brain development and plasticity through CX3CR1-dependent engulfing and phagocytosis of synaptic material and the shaping of synaptic connectivity [91]. Indeed, mice lacking LepRs in CX_3CR1-positive subsets of myeloid cells, including microglia, have reduced POMC-derived, α-MSH-positive nerve terminals in the PVN, and this is associated with less of the phagocytic indicator CD68 in microglia without LepR, indicating that leptin-regulated microglial phagocytosis might be crucial for ARC^{POMC} neural projection to the PVN [92]. Microglia act as sentinels for virtually all neuropathological changes [93]. By virtue of the richness of their surface receptors, microglial cells are able to sense pathogen-associated molecular patterns with Toll-like receptors (TLRs), ATP/ADP (from injured cells) by purinergic receptors and glycocalix-bound sialic acids via sialic acid-binding immunoglobulin-like lectins (Siglecs), as well as cytokines, chemokines, and neurotransmitters [94] by their respective cognate receptors (Figure 2). The ability to differentially sense these various compounds results in region-specific diversity [95].

Given the crucial role that microglia play in hypothalamic plasticity, including regulation of food intake and energy expenditure [96], the detection of their diet-induced chronic activation and eventual loss of function may provide novel cues to understand endocrine disorders. In addition, a recent study reported that TLR2-driven activation of hypothalamic microglia affects POMC neuronal activation and related promotion of sickness behavior [97]. Loss of TLR2-dependent microglia activation alters the GABAergic inputs to POMC neurons and is associated with a body weight loss and anorexia [97].

Overall these recent findings demonstrate that hypothalamic astrocytes, tanycytes and microglia regulate numerous and diverse molecular cascades involved in the control of systemic metabolism. The underlying mechanisms include morphological remodeling that alters neurotransmitter dynamics at the synapse, as well as synaptic connectivity, the release of signaling molecules capable of altering neuronal function and controlling the access of circulating signals into the brain.

Morphological and functional impairment of the hypothalamic cell matrix in obesity and translational aspects

The hypothalamic feeding circuits are highly vulnerable to obesogenic diets. Malfunctioning of basic cellular processes, including autophagy or leptin-induced reactive oxygen species (ROS) formation, disturbs the dynamics of mitochondrial fission and fusion, promotes endoplasmic reticulum (ER) stress and alters the formation of mitochondria-to-ER contacts. These dysfunctions have been observed in diet-induced obese (DIO) mice, particularly in POMC neurons, and all these processes have been reported to contribute to the development of obesity [33,98]. Likewise, DIO-induced dysfunctional activity has been observed in both AgRP and POMC neurons, including altered synaptic input organization associated with a reduced ability to sense important metabolic signals [30,99]. Alterations in dopaminergic brain activation also occur in obese mice, indicating a possible aberrant activation of reward processes that might result in excessive consumption of high-energy food in obese subjects [100].

In hypercaloric DIO, dietary fat, and especially saturated fatty acids, are considered to be the essential components initiating pro-inflammatory responses in the hypothalamus [101]. However, by carefully dissecting the dietary components, it was found that the combination of high-fat and high-carbohydrate content in the diet can stimulate both POMC and NPY/AgRP neurons to produce advanced glycation end-products (AGEs), which are then secreted by the neurons and taken up by microglia [102]. Upon AGE stimulation, microglia become activated and produce more tumor necrosis factor (TNF)-α to enhance microglial immune capacity [103] and mitochondrial stress in POMC neurons [104], which in the long run may result in POMC neuronal dysfunction.

Astrocytes are also susceptible to obesogenic diets, developing a reactive phenotype in the ARC [99]. Changes in the reactivity and/or the distribution of astrocytes in the hypothalamus are associated with synaptic organization and the responsiveness of POMC neurons to metabolic factors including glucose [105] or leptin [74]. These might also affect the content of local synaptic messengers, such as endocannabinoids, and subsequent responses of hypothalamic neurons (*e.g.,* POMC cells) to metabolic hormones [32]. Analogously, the loss of lipid sensing via lipoprotein lipase (LPL) in astrocytes promotes hypothalamic ceramide accumulation and exacerbates body weight gain in response to a hypercaloric diet [106]. Consumption of a hypercaloric diet potentiates the reactivity of astrocytes and also affects the number and size of microglia in the ARC and the ME [107,108] prior to any changes in body weight gain [107], suggesting a potential role of these glial cells in the pathogenesis of obesity (Box 3).

Box 3. Underlying mechanisms linking hypothalamic glia activation and obesity

Using wild-type (wt), monogenic obese *ob/ob*, *db/db* (leptin-receptor mutation), or MC4R KO mice on either a hypercaloric diet or a standardized chow diet, the microglial changes in the ARC have been demonstrated to be due to hormones and diet, rather than to body weight itself [96]. The hypercaloric diet-induced increase in microglial numbers is solely due to their proliferation, as peripheral mononuclear cells were not detected in the hypothalamus [109]. Inhibiting microglial proliferation, by central delivery of the antimitotic drug arabinofuranosyl cytidine, reduces hypothalamic inflammation

and adiposity and restores leptin sensitivity in mice fed a hypercaloric diet [110]. Similarly, depleting microglia with PLX5622, a CSF1R inhibitor, and restraining microglial negative regulator of nuclear factor κB (NF-κB) signaling, abrogates diet-induced hyperphagia and weight gain [111]. On the other hand, microglial-specific deletion of A20, a negative regulator of NF-κB signaling, induces microgliosis, reduced energy expenditure, and consequent weight gain as well as increased food intake without dietary challenge [111]. It is not clear at present as to how far such changes are reversible, but dystrophic microglia have been found in brains of obese humans [109].

The IKKβ/NF-κB pathway in the MBH has been recently identified as a key regulator of astrocytic distal process plasticity, with functional consequences to both acutely and chronically-regulated metabolic parameters [112]. Similar to what has been described in microglia, when IKKβ is selectively knocked out in astrocytes, mice are protected against DIO, and the conditional inactivation of IKKβ in hypothalamic astrocytes in adult mice counteracts the overfeeding induced by a chronic exposure to a hypercaloric diet [112,113].

Other studies demonstrate that in *db/db* and DIO mice, leptin loses its ability to activate LepR-associated signaling pathways (STAT3, Akt and ERK) in tanycytes. In both obesity mouse models, exogenous leptin accumulates in the ME and never reaches the MBH [89]. Together with human data indicating that the transport of leptin into the CSF is dramatically reduced in obese patients [114,115], these findings suggest that the leptin taken up by ME tanycytes in *db/db* and DIO mice is not released into the third ventricle. Activation of the ERK signaling pathway in tanycytes by epidermal growth factor (EGF) rescues leptin translocation from the ME to the MBH in both mouse models, ameliorates the aberrant hypothalamic leptin signaling and improves metabolic status in DIO mice [89]. Finally, glial cells and neurons are not the only cells that are affected as the density and length of microvessels increase in both obese rodents and humans, and there is an accumulation of immunoglobulin G (IgG) that cannot be found elsewhere in the CNS [116,117].

Together these studies demonstrate that the hypothalamus and its specific cells are highly diet-responsive, although the physiological significance of this phenomenon and its contribution to metabolic diseases remain unknown.

Outlook

Functional Dissection of Cellular Heterogeneity in the CNS control of Metabolism

Although considerable effort has been made to better understand the relevant mechanisms underlying the brain control of systemic metabolism, the global obesity epidemic continues to rise. Based on the information and emerging models summarized above, it appears that the successful translation of experimental findings on cellular heterogeneity in the brain's control of body weight, food intake and metabolism from mice to men might lead to improved strategies to fight human metabolic diseases. Specifically, knowledge of the cellular heterogeneity of the CNS control of metabolism might lead to the generation of novel drug candidates with reduced side effects as specific targeting of distinct cell types or subpopulations becomes feasible. Over the last two decades, numerous studies unraveled the

cellular heterogeneity of neurons in metabolic control. Now, the discovery that glia can participate in governing systemic metabolism suggests that similar functional patterns may exist for astrocytes, tanycytes and microglia. Moreover, diet-induced intracellular adaptations in distinct groups of glial cells may offer a targeting potential for new therapeutic strategies that could reverse immunometabolic dysfunction and obesity by protection or modulation of hypothalamic glial function [99]. In addition, future investigations into the biology of other types of glial cells in the hypothalamus, such as oligodendrocytes and NG2 glial cells (also known as oligodendrocyte precursors), which play a role in the maintenance of neuronal processes of sensory neurons in CVOs [13], will undoubtedly improve our understanding of the central control of systemic metabolism. Likewise, further studies unraveling the processes by which metabolic hormones gain access to hypothalamic circuits will shed more light in this regard. Parallel studies in mice and men are required to mechanistically link glial activation and alterations of energy expenditure and weight gain.

Acknowledgement

This work was supported by European Research Council ERC AdG (HypoFlam no. 695054) to MHT and ERC STG (AstroNeuroCrosstalk no. 757393) to CGC; ANR/DFG Nutripathos Project ANR-15-CE14-0030-01/02 to MHT and SL; the Deutsche Forschungsgemeinschaft (SFB 1052) Obesity Mechanisms to MK. and IB; and the Agence National pour la Recherche (ANR) grant number ANR-15-CE14-0025 to VP and ANR-15-CE14-0030-01 and ANR-16-CE14-0026-02 to SL.

Competing interests statement

The authors declare no competing interests.

Figure legends

Figure 1. The cellular functional heterogeneity of hypothalamic AgRP/NPY and POMC neurons in metabolic sensing and systemic metabolism. The arcuate nucleus is the "brain window" of the hypothalamus in that a wide array of metabolic hormones and nutrients are sensed through specific receptors and transporters expressed by AgRP/NPY neurons (A) and POMC neurons (B) in this brain region. These metabolic signals are then integrated and relayed to specific downstream circuits in other hypothalamic and extra-hypothalamic areas involved in metabolic regulation. These areas are located in both forebrain and hindbrain regions. The outputs of these regions control satiety, feeding pattern, energy expenditure, glucose metabolism and insulin sensitivity. Thus, systemic metabolism is controlled by a brain circuit comprised of heterogeneous neuronal populations.
AgRP: agouti-related peptide; BAT: brown adipose tissue; BNST: bed nucleus of the stria terminalis; LH: lateral hypothalamus; NTS: nucleus of the solitary tract; NPY: neuropeptide Y; PBN: parabrachial nucleus; POMC: pro-opiomelanocortin; PVN: paraventricular nucleus of the hypothalamus; PVT: the paraventricular nucleus of the thalamus; ROS: reactive oxygen species.

Figure 2. The cellular functional heterogeneity of hypothalamic non-neuronal cells in metabolic sensing and systemic metabolism.

Tanycytes that line the third ventricle (3rd ventricle) are able to transport leptin and ghrelin from the general circulation into the third ventricle, or carry glucose, leptin and ghrelin from the third ventricle to parenchymal area where key metabolic sensing neurons are located. Astrocytes are also involved in sensing circulating metabolic-associated factors and consequently regulating neighboring neuronal functions. On the other hand, microglia provide a neuroprotective role by secreting neurotrophic factors such as BDNF, engulfing cellular debris. However, when neurons produce excessive debris and metabolic waste in an obesogenic environment, microglia persistently exhibit a pro-inflammatory state. The microglia-derived inflammatory cytokines such as TNF act on neurons resulting in neural damage. Eventually, a vicious cycle is formed between the reactive microglia and hypothalamic neurons, promoting hypothalamic dysfunction and affecting the brain control of systemic energy metabolism.

BDNF: brain-derived neurotrophic factor; TNFα: tumor necrosis factor alpha.

References

1 Bruch, H. The Frohlich syndrome: report of the original case. 1939. *Obes Res* **1**, 329-331 (1993).

2 Hetherington, A. W. Non-production of hypothalamic obesity in the rat by lesions rostral or dorsal to the ventro-medial hypothalamic nuclei. *J Comp Neurol.* **80(1)**, 33–45 (1944).

3 Brobeck, J. R. Mechanism of the development of obesity in animals with hypothalamic lesions. *Physiol Rev* **26**, 541-559 (1946).

4 Hetherington, A. W. R., S.W. . The relation of various hypothalamic lesions to adiposity in the rat. . *J Comp Neurol.* **76(3)**, 475–499 (1942).

5 Anand, B. K. & Brobeck, J. R. Hypothalamic control of food intake in rats and cats. *Yale J Biol Med* **24**, 123-140 (1951).

6 Woods, S. C., Lotter, E. C., McKay, L. D. & Porte, D., Jr. Chronic intracerebroventricular infusion of insulin reduces food intake and body weight of baboons. *Nature* **282**, 503-505 (1979).

7 Zhang, Y. *et al.* Positional cloning of the mouse obese gene and its human homologue. *Nature* **372**, 425-432 (1994).

8 Kojima, M. *et al.* Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature* **402**, 656-660, doi:10.1038/45230 (1999).

9 Huszar, D. *et al.* Targeted disruption of the melanocortin-4 receptor results in obesity in mice. *Cell* **88**, 131-141 (1997).

10 Coleman, D. L. Diabetes-obesity syndromes in mice. *Diabetes* **31**, 1-6 (1982).

11 Luquet, S., Perez, F. A., Hnasko, T. S. & Palmiter, R. D. NPY/AgRP neurons are essential for feeding in adult mice but can be ablated in neonates. *Science* **310**, 683-685, doi:10.1126/science.1115524 (2005).

12 Cone, R. D. Anatomy and regulation of the central melanocortin system. *Nat Neurosci* **8**, 571-578, doi:10.1038/nn1455 (2005).

13 Djogo, T. *et al.* Adult NG2-Glia Are Required for Median Eminence-Mediated Leptin Sensing and Body Weight Control. *Cell Metab* **23**, 797-810, doi:10.1016/j.cmet.2016.04.013 (2016).

14 Tatemoto, K., Carlquist, M. & Mutt, V. Neuropeptide Y--a novel brain peptide with structural similarities to peptide YY and pancreatic polypeptide. *Nature* **296**, 659-660 (1982).

15 Miltenberger, R. J., Mynatt, R. L., Wilkinson, J. E. & Woychik, R. P. The role of the agouti gene in the yellow obese syndrome. *J Nutr* **127**, 1902S-1907S (1997).

16 Gantz, I. *et al.* Molecular cloning of a novel melanocortin receptor. *J Biol Chem* **268**, 8246-8250 (1993).

17 Xu, A. W. *et al.* Effects of hypothalamic neurodegeneration on energy balance. *PLoS Biol* **3**, e415, doi:10.1371/journal.pbio.0030415 (2005).

18 Krashes, M. J. *et al.* Rapid, reversible activation of AgRP neurons drives feeding behavior in mice. *J Clin Invest* **121**, 1424-1428, doi:10.1172/JCI46229 (2011).

19 Wu, Q., Howell, M. P., Cowley, M. A. & Palmiter, R. D. Starvation after AgRP neuron ablation is independent of melanocortin signaling. *Proc Natl Acad Sci U S A* **105**, 2687-2692, doi:10.1073/pnas.0712062105 (2008).

20 Dietrich, M. O., Zimmer, M. R., Bober, J. & Horvath, T. L. Hypothalamic Agrp neurons drive stereotypic behaviors beyond feeding. *Cell* **160**, 1222-1232, doi:10.1016/j.cell.2015.02.024 (2015).

21 Padilla, S. L. *et al.* Agouti-related peptide neural circuits mediate adaptive behaviors in the starved state. *Nat Neurosci* **19**, 734-741, doi:10.1038/nn.4274 (2016).
22 Betley, J. N. *et al.* Neurons for hunger and thirst transmit a negative-valence teaching signal. *Nature* **521**, 180-185, doi:10.1038/nature14416 (2015).
23 Chen, Y., Lin, Y. C., Kuo, T. W. & Knight, Z. A. Sensory detection of food rapidly modulates arcuate feeding circuits. *Cell* **160**, 829-841, doi:10.1016/j.cell.2015.01.033 (2015).
24 Betley, J. N., Cao, Z. F., Ritola, K. D. & Sternson, S. M. Parallel, redundant circuit organization for homeostatic control of feeding behavior. *Cell* **155**, 1337-1350, doi:10.1016/j.cell.2013.11.002 (2013).
25 Steculorum, S. M. *et al.* AgRP Neurons Control Systemic Insulin Sensitivity via Myostatin Expression in Brown Adipose Tissue. *Cell* **165**, 125-138, doi:10.1016/j.cell.2016.02.044 (2016).
26 Wu, Q., Boyle, M. P. & Palmiter, R. D. Loss of GABAergic signaling by AgRP neurons to the parabrachial nucleus leads to starvation. *Cell* **137**, 1225-1234, doi:10.1016/j.cell.2009.04.022 (2009).
27 Joly-Amado, A. *et al.* Hypothalamic AgRP-neurons control peripheral substrate utilization and nutrient partitioning. *EMBO J* **31**, 4276-4288, doi:10.1038/emboj.2012.250 (2012).
28 Matarese, G. *et al.* Hunger-promoting hypothalamic neurons modulate effector and regulatory T-cell responses. *Proc Natl Acad Sci U S A* **110**, 6193-6198, doi:10.1073/pnas.1210644110 (2013).
29 Kim, J. G. *et al.* AgRP Neurons Regulate Bone Mass. *Cell Rep* **13**, 8-14, doi:10.1016/j.celrep.2015.08.070 (2015).
30 Pinto, S. *et al.* Rapid rewiring of arcuate nucleus feeding circuits by leptin. *Science* **304**, 110-115, doi:10.1126/science.1089459 (2004).
31 Varela, L. & Horvath, T. L. AgRP neurons: a switch between peripheral carbohydrate and lipid utilization. *EMBO J* **31**, 4252-4254, doi:10.1038/emboj.2012.287 (2012).
32 Koch, M. *et al.* Hypothalamic POMC neurons promote cannabinoid-induced feeding. *Nature* **519**, 45-50, doi:10.1038/nature14260 (2015).
33 Diano, S. *et al.* Peroxisome proliferation-associated control of reactive oxygen species sets melanocortin tone and feeding in diet-induced obesity. *Nat Med* **17**, 1121-1127, doi:10.1038/nm.2421 (2011).
34 Toda, C., Santoro, A., Kim, J. D. & Diano, S. POMC Neurons: From Birth to Death. *Annu Rev Physiol* **79**, 209-236, doi:10.1146/annurev-physiol-022516-034110 (2017).
35 Lam, B. Y. H. *et al.* Heterogeneity of hypothalamic pro-opiomelanocortin-expressing neurons revealed by single-cell RNA sequencing. *Mol Metab* **6**, 383-392, doi:10.1016/j.molmet.2017.02.007 (2017).
36 Romanov, R. A. *et al.* Molecular interrogation of hypothalamic organization reveals distinct dopamine neuronal subtypes. *Nat Neurosci* **20**, 176-188, doi:10.1038/nn.4462 (2017).
37 Fenselau, H. *et al.* A rapidly acting glutamatergic ARC-->PVH satiety circuit postsynaptically regulated by alpha-MSH. *Nat Neurosci* **20**, 42-51, doi:10.1038/nn.4442 (2017).
38 Zhang, X. & van den Pol, A. N. Hypothalamic arcuate nucleus tyrosine hydroxylase neurons play orexigenic role in energy homeostasis. *Nat Neurosci* **19**, 1341-1347, doi:10.1038/nn.4372 (2016).
39 Fitzgerald, P. & Dinan, T. G. Prolactin and dopamine: what is the connection? A review article. *J Psychopharmacol* **22**, 12-19, doi:10.1177/0269216307087148 (2008).
40 Kong, D. *et al.* GABAergic RIP-Cre neurons in the arcuate nucleus selectively regulate energy expenditure. *Cell* **151**, 645-657, doi:10.1016/j.cell.2012.09.020 (2012).
41 Horvath, T. L., Diano, S. & van den Pol, A. N. Synaptic interaction between hypocretin (orexin) and neuropeptide Y cells in the rodent and primate hypothalamus: a novel circuit implicated in metabolic and endocrine regulations. *J Neurosci* **19**, 1072-1087 (1999).
42 Horvath, T. L. *et al.* Hypocretin (orexin) activation and synaptic innervation of the locus coeruleus noradrenergic system. *J Comp Neurol* **415**, 145-159 (1999).
43 Nieh, E. H. *et al.* Decoding neural circuits that control compulsive sucrose seeking. *Cell* **160**, 528-541, doi:10.1016/j.cell.2015.01.003 (2015).
44 King, B. M. The rise, fall, and resurrection of the ventromedial hypothalamus in the regulation of feeding behavior and body weight. *Physiol Behav* **87**, 221-244, doi:10.1016/j.physbeh.2005.10.007 (2006).
45 Choi, Y. H., Fujikawa, T., Lee, J., Reuter, A. & Kim, K. W. Revisiting the Ventral Medial Nucleus of the Hypothalamus: The Roles of SF-1 Neurons in Energy Homeostasis. *Front Neurosci* **7**, 71, doi:10.3389/fnins.2013.00071 (2013).
46 Kim, K. W. *et al.* Steroidogenic factor 1 directs programs regulating diet-induced thermogenesis and leptin action in the ventral medial hypothalamic nucleus. *Proc Natl Acad Sci U S A* **108**, 10673-10678, doi:10.1073/pnas.1102364108 (2011).

47 Woods, S. C., Seeley, R. J., Porte, D., Jr. & Schwartz, M. W. Signals that regulate food intake and energy homeostasis. *Science* **280**, 1378-1383 (1998).
48 Mountjoy, K. G., Mortrud, M. T., Low, M. J., Simerly, R. B. & Cone, R. D. Localization of the melanocortin-4 receptor (MC4-R) in neuroendocrine and autonomic control circuits in the brain. *Mol Endocrinol* **8**, 1298-1308, doi:10.1210/mend.8.10.7854347 (1994).
49 Balthasar, N. *et al.* Divergence of melanocortin pathways in the control of food intake and energy expenditure. *Cell* **123**, 493-505, doi:10.1016/j.cell.2005.08.035 (2005).
50 Koch, M. & Horvath, T. L. Molecular and cellular regulation of hypothalamic melanocortin neurons controlling food intake and energy metabolism. *Mol Psychiatry* **19**, 752-761, doi:10.1038/mp.2014.30 (2014).
51 Harris, G. C., Wimmer, M. & Aston-Jones, G. A role for lateral hypothalamic orexin neurons in reward seeking. *Nature* **437**, 556-559, doi:10.1038/nature04071 (2005).
52 Castro, D. C. & Berridge, K. C. Advances in the neurobiological bases for food 'liking' versus 'wanting'. *Physiol Behav* **136**, 22-30, doi:10.1016/j.physbeh.2014.05.022 (2014).
53 Fulton, S. *et al.* Leptin regulation of the mesoaccumbens dopamine pathway. *Neuron* **51**, 811-822 (2006).
54 Figlewicz, D. P. Expression of receptors for insulin and leptin in the ventral tegmental area/substantia nigra (VTA/SN) of the rat: Historical perspective. *Brain Res* **1645**, 68-70, doi:10.1016/j.brainres.2015.12.041 (2016).
55 Abizaid, A. *et al.* Ghrelin modulates the activity and synaptic input organization of midbrain dopamine neurons while promoting appetite. *J Clin Invest* **116**, 3229-3239, doi:10.1172/JCI29867 (2006).
56 Woods, S. C. & Begg, D. P. Food for Thought: Revisiting the Complexity of Food Intake. *Cell Metab* **22**, 348-351, doi:10.1016/j.cmet.2015.08.017 (2015).
57 Davidson, T. L. *et al.* Contributions of the hippocampus and medial prefrontal cortex to energy and body weight regulation. *Hippocampus* **19**, 235-252, doi:10.1002/hipo.20499 (2009).
58 Diano, S. *et al.* Ghrelin controls hippocampal spine synapse density and memory performance. *Nat Neurosci* **9**, 381-388, doi:10.1038/nn1656 (2006).
59 Lathe, R. Hormones and the hippocampus. *J Endocrinol* **169**, 205-231 (2001).
60 Carus-Cadavieco, M. *et al.* Gamma oscillations organize top-down signalling to hypothalamus and enable food seeking. *Nature* **542**, 232-236, doi:10.1038/nature21066 (2017).
61 Mandelblat-Cerf, Y. *et al.* Arcuate hypothalamic AgRP and putative POMC neurons show opposite changes in spiking across multiple timescales. *Elife* **4**, doi:10.7554/eLife.07122 (2015).
62 Holtmaat, A. & Svoboda, K. Experience-dependent structural synaptic plasticity in the mammalian brain. *Nat Rev Neurosci* **10**, 647-658, doi:10.1038/nrn2699 (2009).
63 Dietrich, M. O. & Horvath, T. L. Hypothalamic control of energy balance: insights into the role of synaptic plasticity. *Trends Neurosci* **36**, 65-73, doi:10.1016/j.tins.2012.12.005 (2013).
64 Cristino, L. *et al.* Obesity-driven synaptic remodeling affects endocannabinoid control of orexinergic neurons. *Proc Natl Acad Sci U S A* **110**, E2229-2238, doi:10.1073/pnas.1219485110 (2013).
65 Oberheim, N. A., Goldman, S. A. & Nedergaard, M. Heterogeneity of astrocytic form and function. *Methods Mol Biol* **814**, 23-45, doi:10.1007/978-1-61779-452-0_3 (2012).
66 Verkhratsky A, a. N. M. Physiology of Astroglia. *Physiol Rev* **98**, 239–389, doi:10.1152/physrev.00042.2016 (2018).
67 Khakh, B. S. & Sofroniew, M. V. Diversity of astrocyte functions and phenotypes in neural circuits. *Nat Neurosci* **18**, 942-952, doi:10.1038/nn.4043 (2015).
68 Araque, A., Parpura, V., Sanzgiri, R. P. & Haydon, P. G. Tripartite synapses: glia, the unacknowledged partner. *Trends Neurosci* **22**, 208-215 (1999).
69 Araque, A. *et al.* Gliotransmitters travel in time and space. *Neuron* **81**, 728-739, doi:10.1016/j.neuron.2014.02.007 (2014).
70 Verkhratsky, A., Orkand, R. K. & Kettenmann, H. Glial calcium: homeostasis and signaling function. *Physiol Rev* **78**, 99-141 (1998).
71 Rouach, N., Koulakoff, A., Abudara, V., Willecke, K. & Giaume, C. Astroglial metabolic networks sustain hippocampal synaptic transmission. *Science* **322**, 1551-1555, doi:10.1126/science.1164022 (2008).
72 Clasadonte, J., Scemes, E., Wang, Z., Boison, D. & Haydon, P. G. Connexin 43-Mediated Astroglial Metabolic Networks Contribute to the Regulation of the Sleep-Wake Cycle. *Neuron* **95**, 1365-1380 e1365, doi:10.1016/j.neuron.2017.08.022 (2017).
73 Garcia-Caceres, C. *et al.* Astrocytic Insulin Signaling Couples Brain Glucose Uptake with Nutrient Availability. *Cell* **166**, 867-880, doi:10.1016/j.cell.2016.07.028 (2016).

643 74 Kim, J. G. *et al.* Leptin signaling in astrocytes regulates hypothalamic neuronal circuits and
644 feeding. *Nat Neurosci* **17**, 908-910, doi:10.1038/nn.3725 (2014).
645 75 Allard, C. *et al.* Hypothalamic astroglial connexins are required for brain glucose sensing-
646 induced insulin secretion. *J Cereb Blood Flow Metab* **34**, 339-346, doi:10.1038/jcbfm.2013.206
647 (2014).
648 76 Chari, M. *et al.* Glucose transporter-1 in the hypothalamic glial cells mediates glucose sensing
649 to regulate glucose production in vivo. *Diabetes* **60**, 1901-1906, doi:10.2337/db11-0120 (2011).
650 77 Schipper, H. M. Gomori-positive astrocytes: biological properties and implications for
651 neurologic and neuroendocrine disorders. *Glia* **4**, 365-377, doi:10.1002/glia.440040404 (1991).
652 78 Young, J. K. & McKenzie, J. C. GLUT2 immunoreactivity in Gomori-positive astrocytes of
653 the hypothalamus. *J Histochem Cytochem* **52**, 1519-1524, doi:10.1369/jhc.4A6375.2004 (2004).
654 79 Chowen, J. A. *et al.* The role of astrocytes in the hypothalamic response and adaptation to
655 metabolic signals. *Prog Neurobiol* **144**, 68-87, doi:10.1016/j.pneurobio.2016.03.001 (2016).
656 80 Tasker, J. G., Oliet, S. H., Bains, J. S., Brown, C. H. & Stern, J. E. Glial regulation of
657 neuronal function: from synapse to systems physiology. *J Neuroendocrinol* **24**, 566-576,
658 doi:10.1111/j.1365-2826.2011.02259.x (2012).
659 81 Gordon, G. R. *et al.* Norepinephrine triggers release of glial ATP to increase postsynaptic
660 efficacy. *Nat Neurosci* **8**, 1078-1086, doi:10.1038/nn1498 (2005).
661 82 Gordon, G. R. *et al.* Astrocyte-mediated distributed plasticity at hypothalamic glutamate
662 synapses. *Neuron* **64**, 391-403, doi:10.1016/j.neuron.2009.10.021 (2009).
663 83 Yang, L., Qi, Y. & Yang, Y. Astrocytes control food intake by inhibiting AGRP neuron
664 activity via adenosine A1 receptors. *Cell Rep* **11**, 798-807, doi:10.1016/j.celrep.2015.04.002 (2015).
665 84 Prevot, V. *et al.* The versatile tanycyte: a hypothalamic integrator of reproduction and energy
666 metabolism. *Endocr Rev*, doi:10.1210/er.2017-00235 (2018).
667 85 Clasadonte, J. & Prevot, V. The special relationship: glia-neuron interactions in the
668 neuroendocrine hypothalamus. *Nat Rev Endocrinol* **14**, 25-44, doi:10.1038/nrendo.2017.124 (2018).
669 86 Langlet, F. *et al.* Tanycytic VEGF-A boosts blood-hypothalamus barrier plasticity and access
670 of metabolic signals to the arcuate nucleus in response to fasting. *Cell Metab* **17**, 607-617,
671 doi:10.1016/j.cmet.2013.03.004 (2013).
672 87 Schaeffer, M. *et al.* Rapid sensing of circulating ghrelin by hypothalamic appetite-modifying
673 neurons. *Proc Natl Acad Sci U S A* **110**, 1512-1517, doi:10.1073/pnas.1212137110 (2013).
674 88 Banks, W. A., DiPalma, C. R. & Farrell, C. L. Impaired transport of leptin across the blood-
675 brain barrier in obesity. *Peptides* **20**, 1341-1345 (1999).
676 89 Balland, E. *et al.* Hypothalamic tanycytes are an ERK-gated conduit for leptin into the brain.
677 *Cell Metab* **19**, 293-301, doi:10.1016/j.cmet.2013.12.015 (2014).
678 90 Collden, G. *et al.* Neonatal overnutrition causes early alterations in the central response to
679 peripheral ghrelin. *Mol Metab* **4**, 15-24, doi:10.1016/j.molmet.2014.10.003 (2015).
680 91 Kettenmann, H., Kirchhoff, F. & Verkhratsky, A Microglia: new roles for the synaptic
681 stripper. *Neuron* **77**, 10-18 (2013).
682 92 Gao, Y. *et al.* Deficiency of leptin receptor in myeloid cells disrupts hypothalamic metabolic
683 circuits and causes body weight increase. *Mol Metab* **7**, 155-160, doi:10.1016/j.molmet.2017.11.003
684 (2018).
685 93 Salter, M. W. & Stevens, B. Microglia emerge as central players in brain disease. *Nat Med* **23**,
686 1018-1027, doi:10.1038/nm.4397 (2017).
687 94 Kettenmann, H., Hanisch, U.K., Noda, M. & Verkhratsky, A Physiology of microglia. *Physiol*
688 *Rev* **91**, 461-553 (2011).
689 95 Grabert, K. *et al.* Microglial brain region-dependent diversity and selective regional
690 sensitivities to aging. *Nat Neurosci* **19**, 504-516, doi:10.1038/nn.4222 (2016).
691 96 Gao, Y. *et al.* Hormones and diet, but not body weight, control hypothalamic microglial
692 activity. *Glia* **62**, 17-25, doi:10.1002/glia.22580 (2014).
693 97 Jin, S. *et al.* Hypothalamic TLR2 triggers sickness behavior via a microglia-neuronal axis. *Sci*
694 *Rep* **6**, 29424, doi:10.1038/srep29424 (2016).
695 98 Schneeberger, M. *et al.* Mitofusin 2 in POMC neurons connects ER stress with leptin
696 resistance and energy imbalance. *Cell* **155**, 172-187, doi:10.1016/j.cell.2013.09.003 (2013).
697 99 Horvath, T. L. *et al.* Synaptic input organization of the melanocortin system predicts diet-
698 induced hypothalamic reactive gliosis and obesity. *Proc Natl Acad Sci U S A* **107**, 14875-14880,
699 doi:10.1073/pnas.1004282107 (2010).
700 100 Stoeckel, L. E. *et al.* Widespread reward-system activation in obese women in response to
701 pictures of high-calorie foods. *Neuroimage* **41**, 636-647, doi:10.1016/j.neuroimage.2008.02.031
702 (2008).

101 Milanski, M. *et al.* Saturated fatty acids produce an inflammatory response predominantly through the activation of TLR4 signaling in hypothalamus: implications for the pathogenesis of obesity. *J Neurosci* **29**, 359-370 (2009).
102 Gao, Y. *et al.* Dietary sugars, not lipids, drive hypothalamic inflammation. *Mol Metab* **6**, 897-908, doi:10.1016/j.molmet.2017.06.008 (2017).
103 Kuno, R. *et al.* Autocrine activation of microglia by tumor necrosis factor-alpha. *J Neuroimmunol* **162**, 89-96, doi:10.1016/j.jneuroim.2005.01.015 (2005).
104 Yi, C. X. *et al.* TNFalpha drives mitochondrial stress in POMC neurons in obesity. *Nat Commun* **8**, 15143, doi:10.1038/ncomms15143 (2017).
105 Fuente-Martin, E. *et al.* Leptin regulates glutamate and glucose transporters in hypothalamic astrocytes. *J Clin Invest* **122**, 3900-3913, doi:10.1172/JCI64102 (2012).
106 Gao, Y. *et al.* Disruption of Lipid Uptake in Astroglia Exacerbates Diet Induced Obesity. *Diabetes*, doi:10.2337/db16-1278 (2017).
107 Thaler, J. P. *et al.* Obesity is associated with hypothalamic injury in rodents and humans. *J Clin Invest* **122**, 153-162, doi:10.1172/JCI59660 (2012).
108 Balland, E. & Cowley, M. A. Short-term high fat diet increases the presence of astrocytes in the hypothalamus of C57BL6 mice without altering leptin sensitivity. *J Neuroendocrinol*, doi:10.1111/jne.12504 (2017).
109 Baufeld, C., Osterloh, A., Prokop, S., Miller, K. R. & Heppner, F. L. High-fat diet-induced brain region-specific phenotypic spectrum of CNS resident microglia. *Acta Neuropathol*, doi:10.1007/s00401-016-1595-4 (2016).
110 Andre, C. *et al.* Inhibiting Microglia Expansion Prevents Diet-Induced Hypothalamic and Peripheral Inflammation. *Diabetes* **66**, 908-919, doi:10.2337/db16-0586 (2017).
111 Valdearcos, M. *et al.* Microglial Inflammatory Signaling Orchestrates the Hypothalamic Immune Response to Dietary Excess and Mediates Obesity Susceptibility. *Cell Metab* **26**, 185-197 e183, doi:10.1016/j.cmet.2017.05.015 (2017).
112 Zhang, Y., Reichel, J. M., Han, C., Zuniga-Hertz, J. P. & Cai, D. Astrocytic Process Plasticity and IKKbeta/NF-kappaB in Central Control of Blood Glucose, Blood Pressure, and Body Weight. *Cell Metab* **25**, 1091-1102 e1094, doi:10.1016/j.cmet.2017.04.002 (2017).
113 Douglass, J. D., Dorfman, M. D., Fasnacht, R., Shaffer, L. D. & Thaler, J. P. Astrocyte IKKbeta/NF-kappaB signaling is required for diet-induced obesity and hypothalamic inflammation. *Mol Metab* **6**, 366-373, doi:10.1016/j.molmet.2017.01.010 (2017).
114 Caro, J. F. *et al.* Decreased cerebrospinal-fluid/serum leptin ratio in obesity: a possible mechanism for leptin resistance. *Lancet* **348**, 159-161 (1996).
115 Schwartz, M. W., Peskind, E., Raskind, M., Boyko, E. J. & Porte, D., Jr. Cerebrospinal fluid leptin levels: relationship to plasma levels and to adiposity in humans. *Nat Med* **2**, 589-593 (1996).
116 Yi, C. X. *et al.* High calorie diet triggers hypothalamic angiopathy. *Mol Metab* **1**, 95-100, doi:10.1016/j.molmet.2012.08.004 (2012).
117 Yi, C. X., Tschop, M. H., Woods, S. C. & Hofmann, S. M. High-fat-diet exposure induces IgG accumulation in hypothalamic microglia. *Dis Model Mech* **5**, 686-690, doi:10.1242/dmm.009464 (2012).