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1 **Role of astrocytes, microglia and tanyocytes in brain control of systemic metabolism**

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40 **Abstract**

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

50

51 **Introduction**

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

63 **Focus on the hypothalamus**

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

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

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

79 **Hypothalamic neuronal networks**

80 **Arcuate nucleus**

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

93

94 ***NPY/AgRP neurons – linking metabolism with behavior***

95 The discovery of the co-expression of two potent orexigenic peptides, NPY and AgRP, within the same
96 ARC neurons provided compelling evidence that these cells are the long-sought hunger neurons. New
97 methodologies, including cell-type specific ablation and acute modulation of neuronal activity
98 combined with tracing methods, have provided detailed views of how these neurons influence both
99 feeding and non-feeding-related behaviors. Ablation of AgRP neurons leads to starvation and death
100 ^{11,17}, while optogenetic or pharmacogenetic activation or inhibition ¹⁸ of AgRP neurons *in vivo*
101 positively or negatively affects feeding, respectively. Although AgRP antagonizes melanocortin (MC)
102 receptors, this may not be its underlying mechanism since acute stimulation or ablation of AgRP
103 rapidly affects feeding during tonic inhibition of the MC system, implying that AgRP neurons act
104 independently of acute MC signaling ¹⁹. When food is available, activating the soma of AgRP neurons
105 increases food consumption, whereas the same stimulation in the absence of food increases stereotypic
106 and compulsive behaviors ²⁰. Optogenetic or pharmacogenetic excitation of AgRP neurons – as a proxy
107 for what occurs in food deprivation – revealed that, depending on the experimental context and food
108 availability, activation of these cells can promote adaptive responses facilitating escape from the state
109 of deprivation. Then, when food is discovered, this behavior can immediately revert, indicating that
110 AgRP neurons have the ability to rapidly respond to food-related cues rather than to calories *per se* ²¹.
111 Indeed, *in vivo* calcium imaging of AgRP neurons in freely moving animals has demonstrated their
112 rapid reductions in activity upon detection of food cues, even before substantial calories are
113 consumed ^{22,23}.

114

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

134

135 ***POMC neurons: center of body weight regulation***

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

154

155 POMC neurons project to different brain areas (Figure 1B), with neurons in the rostral ARC projecting
156 mainly to autonomic brainstem regions and neurons in the caudal ARC primarily connecting with other
157 hypothalamic nuclei such as the PVN³⁴. The region-specific distribution of ARC POMC neurons also
158 determines their responsiveness to metabolic factors, with those in the medial ARC being
159 predominantly sensitive to glucose, while those in the lateral ARC are more responsive to leptin³⁵.
160 Although most POMC cells have functionally active leptin receptors (LepRs), those which do are not
161 more responsive to insulin. One population of POMC cells is insensitive to both leptin and insulin but
162 is activated by serotonin or estrogen³⁴. In sum, POMC neurons represent a heterogeneous population
163 that orchestrates fundamental functions in the maintenance of energy homeostasis, including the
164 regulation of feeding behavior and energy expenditure and maintenance of blood glucose levels.

165

166 **Other key hypothalamic neuronal populations**

167 Numerous neuronal populations in the ARC and other hypothalamic nuclei are required for the
168 physiological control of energy metabolism³⁶. One subpopulation of ARC neurons expresses oxytocin
169 receptors and releases glutamate, but does not contain POMC. Rather, it promotes rapid onset of
170 satiation by activating ARC-to-PVN projections³⁷. Orexigenic dopaminergic (DA) neurons that
171 reciprocally control the activity of nearby AgRP and POMC cells have recently been characterized in
172 the ARC³⁸. Ghrelin triggers increased activity of these ARC DA neurons through affecting AgRP and
173 POMC neurons. In addition to their acute effects on feeding, ARC DA neurons inhibit lactotrophs in
174 the anterior pituitary, preventing lactation³⁹. In RIP-Cre transgenic mice, the neurons expressing Cre
175 (RIP-Cre neurons) are located throughout the brain including the ARC but are distinct from POMC and
176 AgRP neurons. RIP-Cre neurons exert GABAergic inhibition onto PVN neurons and mediate increased
177 energy expenditure, but do not decrease feeding associated with leptin⁴⁰.

178

179 Other hypothalamic nuclei also contain specific neuronal populations that participate in the regulation
180 of energy metabolism. In the LH, hypocretin/orexin-expressing glutamatergic neurons project to
181 several hypothalamic nuclei and other brain areas, promoting arousal and food intake^{41,42}. Neighboring
182 LH melanin-concentrating hormone (MCH) neurons are GABAergic and elicit hyperphagia. Finally,
183 GABAergic projections from LH neurons to the ventral tegmental area (VTA) drive vigorous feeding
184⁴³. In contrast to the orexigenic nature of the LH, the VMH primarily promotes reduced food
185 consumption and consists of heterogeneous subtypes of neurons expressing leptin receptors (LepRs),
186 estrogen receptor α and/or receptors for brain-derived neurotrophic factor (BDNF)⁴⁴. Steroidogenic
187 factor-1 (SF-1) is a well-known cell marker that, in the brain, is selectively produced by neurons in the
188 VMH⁴⁵. These neurons regulate thermogenesis and promote leptin's effects on energy expenditure;
189 malfunctioning of SF-1 neurons is related to the onset of obesity⁴⁶.

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

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

196

197 **Box 1. Connectivity with extra-hypothalamic metabolic control circuits**

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

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

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

224

225 **Box 2. Synaptic plasticity in the hypothalamic regulation of metabolism**

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

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

239

240 **Glia**

241 **Astrocytes: active members of the circuit**

242 Astrocytes are highly diverse in their morphological appearance, functional properties and distribution,
243 both among and within different brain regions ^{65,66}. The majority of protoplasmic astrocytes in the CNS
244 do not have the classic textbook stellate morphology; rather, they resemble “sponges” because of their
245 highly elaborated, plastic and dynamic terminal process arborization ⁶⁷. Astrocytes sense synaptic
246 activity through the expression of neurotransmitter receptors, transporters and ion channels, enabling
247 them to control the concentrations of ions, neurotransmitters and neurohormones in the extracellular
248 space; astrocytes also supply adjacent neurons with glutamine, the obligatory precursor for glutamate
249 and GABA. Astroglia are secretory cells (the gliocrine system), releasing numerous neuroactive
250 molecules including classical neurotransmitters ⁶⁶. The bidirectional communication between astrocytes
251 and neurons was initially embodied in the concept of the tripartite synapse ⁶⁸, that evolved into the
252 multipartite synaptic cradle that includes both astrocytes and microglia as integral elements of the
253 synaptic formation ⁶⁶. Astrocytes modulate neuronal activity and synaptic transmission in several brain
254 areas ⁶⁹ and support synaptogenesis, synaptic maturation, maintenance and extinction. Astroglial cells
255 are organized into networks where extensive communication occurs through gap-junction channels that
256 mediate intercellular signaling in the form of Ca²⁺ or Na⁺ waves ⁷⁰, as well as activity-dependent
257 glucose delivery and trafficking of glucose metabolites from capillaries to distal neurons ⁷¹ in the CNS,
258 including the hypothalamus ⁷². Hypothalamic astrocytes have been reported to play a critical role in
259 regulating nutrient and hormone sensing within the CNS ^{73,74}.

260

261 *Hypothalamic astrocytes are highly glucose-responsive*

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

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

277

278 The phenotype and functionality of hypothalamic astrocytes is determined by adjacent neurons and the
279 local microenvironment; thus, astrocytic activity might change in response to the energetic
280 needs/demands of their surrounding extracellular space. Stimulation of the PVN with norepinephrine or
281 glutamate stimulates astroglial ATP release, leading to the enhancement of synaptic efficacy at
282 glutamatergic synapses ^{81,82}. Astrocytes can act as metabolic sensors regulating food intake by rewiring
283 hypothalamic circuits to ultimately balance energy metabolism via such signals ^{74,83}. MBH astrocytes
284 alter the firing rate of AgRP neurons for the bidirectional regulation of leptin- and ghrelin- regulated
285 feeding circuits via release of adenosine and activation of adenosine A₁ receptors ⁸³ (Figure 2). In
286 addition MBH astrocytes express functional leptin and insulin receptors. Selective genetic loss of these
287 receptors in adult mice reduces the ability of leptin and glucose to suppress food intake ^{73,74}.

288

289 **Tanycytes and the blood-brain barrier (BBB)**

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

302

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

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

321

322 **Microglia and hypothalamic immunity**

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

336

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

343

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

349

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

352 The hypothalamic feeding circuits are highly vulnerable to obesogenic diets. Malfunctioning of basic
353 cellular processes, including autophagy or leptin-induced reactive oxygen species (ROS) formation,
354 disturbs the dynamics of mitochondrial fission and fusion, promotes endoplasmic reticulum (ER) stress
355 and alters the formation of mitochondria-to-ER contacts. These dysfunctions have been observed in
356 diet-induced obese (DIO) mice, particularly in POMC neurons, and all these processes have been
357 reported to contribute to the development of obesity^{33,98}. Likewise, DIO-induced dysfunctional activity
358 has been observed in both AgRP and POMC neurons, including altered synaptic input organization
359 associated with a reduced ability to sense important metabolic signals^{30,99}. Alterations in dopaminergic
360 brain activation also occur in obese mice, indicating a possible aberrant activation of reward processes
361 that might result in excessive consumption of high-energy food in obese subjects¹⁰⁰.

362

363 In hypercaloric DIO, dietary fat, and especially saturated fatty acids, are considered to be the essential
364 components initiating pro-inflammatory responses in the hypothalamus¹⁰¹. However, by carefully
365 dissecting the dietary components, it was found that the combination of high-fat and high-carbohydrate
366 content in the diet can stimulate both POMC and NPY/AgRP neurons to produce advanced glycation
367 end-products (AGEs), which are then secreted by the neurons and taken up by microglia¹⁰². Upon
368 AGE stimulation, microglia become activated and produce more tumor necrosis factor (TNF)- α
369 to enhance microglial immune capacity¹⁰³ and mitochondrial stress in POMC neurons¹⁰⁴, which in the
370 long run may result in POMC neuronal dysfunction.

371

372 Astrocytes are also susceptible to obesogenic diets, developing a reactive phenotype in the ARC⁹⁹.
373 Changes in the reactivity and/or the distribution of astrocytes in the hypothalamus are associated with
374 synaptic organization and the responsiveness of POMC neurons to metabolic factors including glucose
375¹⁰⁵ or leptin⁷⁴. These might also affect the content of local synaptic messengers, such as
376 endocannabinoids, and subsequent responses of hypothalamic neurons (*e.g.*, POMC cells) to metabolic
377 hormones³². Analogously, the loss of lipid sensing via lipoprotein lipase (LPL) in astrocytes promotes
378 hypothalamic ceramide accumulation and exacerbates body weight gain in response to a hypercaloric
379 diet¹⁰⁶. Consumption of a hypercaloric diet potentiates the reactivity of astrocytes and also affects the
380 number and size of microglia in the ARC and the ME^{107,108} prior to any changes in body weight gain
381¹⁰⁷, suggesting a potential role of these glial cells in the pathogenesis of obesity (Box 3).

382

383 **Box 3. Underlying mechanisms linking hypothalamic glia activation and obesity**

384 Using wild-type (wt), monogenic obese *ob/ob*, *db/db* (leptin-receptor mutation), or MC4R KO mice on
385 either a hypercaloric diet or a standardized chow diet, the microglial changes in the ARC have been
386 demonstrated to be due to hormones and diet, rather than to body weight itself⁹⁶. The hypercaloric
387 diet-induced increase in microglial numbers is solely due to their proliferation, as peripheral
388 mononuclear cells were not detected in the hypothalamus¹⁰⁹. Inhibiting microglial proliferation, by
389 central delivery of the antimetabolic drug arabinofuranosyl cytidine, reduces hypothalamic inflammation

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

397 The IKK β /NF- κ B pathway in the MBH has been recently identified as a key regulator of astrocytic
398 distal process plasticity, with functional consequences to both acutely and chronically-regulated
399 metabolic parameters ¹¹². Similar to what has been described in microglia, when IKK β is selectively
400 knocked out in astrocytes, mice are protected against DIO, and the conditional inactivation of IKK β in
401 hypothalamic astrocytes in adult mice counteracts the overfeeding induced by a chronic exposure to a
402 hypercaloric diet ^{112,113}.

403
404 Other studies demonstrate that in *db/db* and DIO mice, leptin loses its ability to activate LepR-
405 associated signaling pathways (STAT3, Akt and ERK) in tanycytes. In both obesity mouse models,
406 exogenous leptin accumulates in the ME and never reaches the MBH ⁸⁹. Together with human data
407 indicating that the transport of leptin into the CSF is dramatically reduced in obese patients ^{114,115}, these
408 findings suggest that the leptin taken up by ME tanycytes in *db/db* and DIO mice is not released into
409 the third ventricle. Activation of the ERK signaling pathway in tanycytes by epidermal growth factor
410 (EGF) rescues leptin translocation from the ME to the MBH in both mouse models, ameliorates the
411 aberrant hypothalamic leptin signaling and improves metabolic status in DIO mice ⁸⁹. Finally, glial
412 cells and neurons are not the only cells that are affected as the density and length of microvessels
413 increase in both obese rodents and humans, and there is an accumulation of immunoglobulin G (IgG)
414 that cannot be found elsewhere in the CNS ^{116,117}.

415
416 Together these studies demonstrate that the hypothalamus and its specific cells are highly diet-
417 responsive, although the physiological significance of this phenomenon and its contribution to
418 metabolic diseases remain unknown.

419

420 **Outlook**

421 **Functional Dissection of Cellular Heterogeneity in the CNS control of Metabolism**

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

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

442

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449 **Competing interests statement**

450

451 The authors declare no competing interests.

452

453 **Figure legends**

454 **Figure 1. The cellular functional heterogeneity of hypothalamic AgRP/NPY and POMC neurons**
455 **in metabolic sensing and systemic metabolism.** The arcuate nucleus is the “brain window” of the
456 hypothalamus in that a wide array of metabolic hormones and nutrients are sensed through specific
457 receptors and transporters expressed by AgRP/NPY neurons (A) and POMC neurons (B) in this brain
458 region. These metabolic signals are then integrated and relayed to specific downstream circuits in other
459 hypothalamic and extra-hypothalamic areas involved in metabolic regulation. These areas are located
460 in both forebrain and hindbrain regions. The outputs of these regions control satiety, feeding pattern,
461 energy expenditure, glucose metabolism and insulin sensitivity. Thus, systemic metabolism is
462 controlled by a brain circuit comprised of heterogeneous neuronal populations.

463 AgRP: agouti-related peptide; BAT: brown adipose tissue; BNST: bed nucleus of the stria terminalis;
464 LH: lateral hypothalamus; NTS: nucleus of the solitary tract; NPY: neuropeptide Y; PBN: parabrachial
465 nucleus; POMC: pro-opiomelanocortin; PVN: paraventricular nucleus of the hypothalamus; PVT: the
466 paraventricular nucleus of the thalamus; ROS: reactive oxygen species.

467

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

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

481

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