

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

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

4  
5 <sup>1</sup>Helmholtz Diabetes Center, Munich, Technische Universität München & German Center for Diabetes  
6 Research.

7 <sup>2</sup>Department of Physiology, Biomedicine Discovery Institute, Monash University, Clayton, VIC 3800,  
8 Australia

9 <sup>3</sup>Inserm, Laboratory of Development and Plasticity of the Neuroendocrine Brain, Centre de recherche  
10 Jean-Pierre Aubert, UMR\_S1172, bâtiment Biserte, 1, place de Verdun 59045 Lille Cedex, France -  
11 Université de Lille, Faculté de Médecine, Lille, France.

12 <sup>4</sup>Univ Paris Diderot, Sorbonne Paris Cité, CNRS UMR 8251, F-75205, Paris, France.

13 <sup>5</sup>Department of Psychiatry and Behavioral Neuroscience, University of Cincinnati, 2170 Galbraith  
14 Avenue, Cincinnati, OH 45237, USA.

15 <sup>6</sup>Institute of Anatomy, Leipzig University, Leipzig, Germany.

16 <sup>7</sup>Department of Comparative Medicine, Yale University School of Medicine, New Haven, CT, 06510,  
17 USA.

18 <sup>8</sup>Department of Endocrinology and Metabolism, Academic Medical Center Amsterdam, 1105  
19 Amsterdam, The Netherlands.

20 <sup>9</sup>Department of Endocrinology, Hospital Infantil Universitario Niño Jesús, Instituto de Investigación la  
21 Princesa; IMDEA Food Institute, CEI UAM + CSIC; CIBEROBN, Instituto Carlos III Madrid, Madrid,  
22 Spain.

23 <sup>10</sup>Faculty of Biology, Medicine and Health, The University of Manchester, Manchester, UK.

24 <sup>11</sup>Achucarro Center for Neuroscience, IKERBASQUE, Basque Foundation for Science, 48011 Bilbao,  
25 Spain.

26 <sup>12</sup>Department of Neuroscience, University of Minnesota, Minneapolis, MN, USA.

27  
28  
29  
30 \*Author for correspondence (tschoep@helmholtz-muenchen.de)

31 Prof. Dr. Matthias H. Tschöp, M.D.

32 Institute for Diabetes and Obesity

33 Helmholtz Center Munich

34 Parkring 13

35 85748 Garching-Hochbrück, Germany.

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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 <sup>1</sup> or by lesions to specific hypothalamic regions,  
66 including the ventromedial (VMH), dorsomedial (DMH) and paraventricular (PVN) nuclei <sup>2-4</sup>, 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) <sup>5</sup>, 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 <sup>6</sup>, leptin <sup>7</sup> and ghrelin <sup>8</sup>  
72 act at the hypothalamus, together with the implementation of genetically-engineered rodent models of  
73 obesity and diabetes <sup>9,10</sup> and cell-specific ablation studies <sup>11</sup>, 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: <sup>12</sup>).

## 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 <sup>13</sup> 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) <sup>14</sup> and  
91 Agouti-related peptide (AgRP) <sup>15</sup>, and the anorexigenic properties of  $\alpha$ -melanocyte-stimulating  
92 hormone ( $\alpha$ -MSH), the post-translational product of proopiomelanocortin (POMC) <sup>16</sup>.

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 <sup>11,17</sup>, while optogenetic or pharmacogenetic activation or inhibition <sup>18</sup> 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 <sup>19</sup>. 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 <sup>20</sup>. 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* <sup>21</sup>.  
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 <sup>22,23</sup>.

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<sup>AgRP</sup>→PVN), to the anterior division of the bed nucleus of the stria terminalis (aBNST),  
119 to the paraventricular thalamus (PVT), or to the LH <sup>24</sup>, 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 <sup>25</sup>. ARC<sup>AgRP</sup> to LH projections promote feeding and insulin resistance through modulation  
123 of brown adipose tissue (BAT) metabolism, and ARC<sup>AgRP</sup> to aBNST<sup>vl/dm</sup> projections coherently but  
124 independently regulate feeding and stimulate expression of muscle-related genes in BAT in addition to  
125 glucose uptake <sup>25</sup>. 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 <sup>24</sup>. Accordingly, genetic or pharmacologic inhibition of AgRP fibers projecting to the PBN  
128 prevents the starvation that results from ablation of all AgRP neurons <sup>26</sup>. 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 <sup>27</sup>, adaptive immune responses and T cell  
133 maturation <sup>28</sup>, and the norepinephrine-dependent control of bone mass <sup>29</sup>.

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 <sup>30</sup>. During a meal, POMC neurons become activated, leading to gradual  
140 onset of satiation and increased energy expenditure <sup>31</sup>. 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 <sup>22,23</sup>. 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 <sup>23</sup>. Activation of POMC  
144 neurons results in the release of several POMC-derived peptides including  $\alpha$ -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 <sup>32</sup>. ARC POMC neurons also secrete  $\beta$ -  
147 endorphin, which, when acutely released into the PVN, triggers feeding in response to cannabinoids in  
148 sated mice <sup>32</sup>. 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 <sup>33</sup>.

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 PVN<sup>34</sup>. 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 leptin<sup>35</sup>.  
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 estrogen<sup>34</sup>. 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 metabolism<sup>36</sup>. 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 projections<sup>37</sup>. Orexigenic dopaminergic (DA) neurons that  
171 reciprocally control the activity of nearby AgRP and POMC cells have recently been characterized in  
172 the ARC<sup>38</sup>. 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 lactation<sup>39</sup>. 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 leptin<sup>40</sup>.

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<sup>41,42</sup>. 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  
184<sup>43</sup>. 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  $\alpha$  and/or receptors for brain-derived neurotrophic factor (BDNF)<sup>44</sup>. Steroidogenic  
187 factor-1 (SF-1) is a well-known cell marker that, in the brain, is selectively produced by neurons in the  
188 VMH<sup>45</sup>. These neurons regulate thermogenesis and promote leptin's effects on energy expenditure;  
189 malfunctioning of SF-1 neurons is related to the onset of obesity<sup>46</sup>.

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 <sup>47</sup>. The brainstem has a high level  
201 of MC4R expression <sup>48</sup>, 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 <sup>49,50</sup>. 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 <sup>51</sup>. These  
211 dopaminergic pathways potently alter motivated “wanting” for the food reward <sup>52</sup> and are directly  
212 impacted by leptin <sup>53</sup>, insulin <sup>54</sup> and ghrelin <sup>55</sup>. 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 <sup>56</sup>. In fact, lesions in the hippocampus promote hyperphagia and adipose  
216 deposition <sup>57</sup>. 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 <sup>58,59</sup>. 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 <sup>60</sup>. 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 <sup>22,23,61</sup>.

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 <sup>62</sup>. 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 <sup>63</sup>. 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 <sup>30</sup>. 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 <sup>64</sup>.

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 <sup>65,66</sup>. 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 <sup>67</sup>. 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 <sup>66</sup>. The bidirectional communication between astrocytes  
251 and neurons was initially embodied in the concept of the tripartite synapse <sup>68</sup>, that evolved into the  
252 multipartite synaptic cradle that includes both astrocytes and microglia as integral elements of the  
253 synaptic formation <sup>66</sup>. Astrocytes modulate neuronal activity and synaptic transmission in several brain  
254 areas <sup>69</sup> 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 Ca<sup>2+</sup> or Na<sup>+</sup> waves <sup>70</sup>, as well as activity-dependent  
257 glucose delivery and trafficking of glucose metabolites from capillaries to distal neurons <sup>71</sup> in the CNS,  
258 including the hypothalamus <sup>72</sup>. Hypothalamic astrocytes have been reported to play a critical role in  
259 regulating nutrient and hormone sensing within the CNS <sup>73,74</sup>.

260

261 *Hypothalamic astrocytes are highly glucose-responsive*

262 Astrocytes play a key role in the transport and sensing of glucose via gap junctions <sup>75</sup>, the astroglial  
263 glucose transporter (GLUT)-1 <sup>76</sup>, and the insulin receptor <sup>73</sup>. 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 <sup>73</sup>. The loss of connexin 43 in astrocytes inhibits wake-  
267 promoting LH orexin neurons by impairing glucose and lactate trafficking through astrocytic networks  
268 <sup>72</sup>. 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 <sup>77</sup>. 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 <sup>78</sup>. 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 <sup>79,80</sup>.

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 <sup>81,82</sup>. Astrocytes can act as metabolic sensors regulating food intake by rewiring  
283 hypothalamic circuits to ultimately balance energy metabolism via such signals <sup>74,83</sup>. 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 A<sub>1</sub> receptors <sup>83</sup> (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 <sup>73,74</sup>.

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 <sup>84</sup>. Although  
292 tanycytes share many common features with astrocytes, they display a unique morphology and distinct  
293 functional characteristics <sup>85</sup>. 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 <sup>84</sup>. 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 <sup>84</sup>.

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) <sup>84</sup>. 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 <sup>84</sup>. 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 <sup>86,87</sup> (Figure 2). Previous studies  
314 suggested that metabolic hormones enter the brain mainly by transport across the vascular endothelium  
315 <sup>88</sup>. 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 <sup>89</sup>. 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) <sup>89</sup>.  
320 Likewise, circulating ghrelin is also transported to the CSF by tanycytes (Figure 2) <sup>90</sup>.

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 <sup>91</sup>. Indeed, mice lacking LepRs in CX<sub>3</sub>CR1-positive subsets of myeloid cells,  
327 including microglia, have reduced POMC-derived,  $\alpha$ -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<sup>POMC</sup> neural projection to the PVN <sup>92</sup>.  
330 Microglia act as sentinels for virtually all neuropathological changes <sup>93</sup>. 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 bound sialic acids via sialic acid-binding immunoglobulin-like lectins (Siglecs), as well as cytokines,  
334 chemokines, and neurotransmitters <sup>94</sup> by their respective cognate receptors (Figure 2). The ability to  
335 differentially sense these various compounds results in region-specific diversity <sup>95</sup>.

336

337 Given the crucial role that microglia play in hypothalamic plasticity, including regulation of food  
338 intake and energy expenditure <sup>96</sup>, 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 <sup>97</sup>. Loss of TLR2-dependent microglia activation alters the  
342 GABAergic inputs to POMC neurons and is associated with a body weight loss and anorexia <sup>97</sup>.

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<sup>33,98</sup>. 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<sup>30,99</sup>. 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 subjects<sup>100</sup>.

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 hypothalamus<sup>101</sup>. 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 microglia<sup>102</sup>. Upon  
368 AGE stimulation, microglia become activated and produce more tumor necrosis factor (TNF)- $\alpha$   
369 to enhance microglial immune capacity<sup>103</sup> and mitochondrial stress in POMC neurons<sup>104</sup>, 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 ARC<sup>99</sup>.  
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  
375<sup>105</sup> or leptin<sup>74</sup>. 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 hormones<sup>32</sup>. 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 diet<sup>106</sup>. 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<sup>107,108</sup> prior to any changes in body weight gain  
381<sup>107</sup>, 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 itself<sup>96</sup>. 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 hypothalamus<sup>109</sup>. 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 <sup>110</sup>. Similarly, depleting  
391 microglia with PLX5622, a CSF1R inhibitor, and restraining microglial negative regulator of nuclear  
392 factor  $\kappa$ B (NF- $\kappa$ B) signaling, abrogates diet-induced hyperphagia and weight gain <sup>111</sup>. On the other  
393 hand, microglial-specific deletion of A20, a negative regulator of NF- $\kappa$ B signaling, induces  
394 microgliosis, reduced energy expenditure, and consequent weight gain as well as increased food intake  
395 without dietary challenge <sup>111</sup>. 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 <sup>109</sup>.

397 The IKK $\beta$ /NF- $\kappa$ 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 <sup>112</sup>. Similar to what has been described in microglia, when IKK $\beta$  is selectively  
400 knocked out in astrocytes, mice are protected against DIO, and the conditional inactivation of IKK $\beta$  in  
401 hypothalamic astrocytes in adult mice counteracts the overfeeding induced by a chronic exposure to a  
402 hypercaloric diet <sup>112,113</sup>.

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 <sup>89</sup>. Together with human data  
407 indicating that the transport of leptin into the CSF is dramatically reduced in obese patients <sup>114,115</sup>, 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 <sup>89</sup>. 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 <sup>116,117</sup>.

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 <sup>99</sup>. 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 <sup>13</sup>, 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

#### 443 **Acknowledgement**

444 This work was supported by European Research Council ERC AdG (HypoFlam no. 695054) to MHT  
445 and ERC STG (AstroNeuroCrosstalk no. 757393) to CGC; ANR/DFG Nutripathos Project ANR-15-  
446 CE14-0030-01/02 to MHT and SL; the Deutsche Forschungsgemeinschaft (SFB 1052) Obesity  
447 Mechanisms to MK. and IB; and the Agence National pour la Recherche (ANR) grant number ANR-  
448 15-CE14-0025 to VP and ANR-15-CE14-0030-01 and ANR-16-CE14-0026-02 to SL.

#### 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 (3<sup>rd</sup> 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 $\alpha$ : tumor necrosis factor alpha.

481

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