Microgliosis: a double-edged sword in the control of food intake

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Abbreviations:

AD: Alzheimer disease

AGEs: advanced glycation end products

AMP: adenosine monophosphate

AN: anorexia nervosa

Anx/anx*:* anorexia gene

Arg1: arginase gene

ATP: adenosine triphosphate

BDNF: brain-derived neurotrophic factor

BrdU: bromo-deoxyuridine

CD11b, CD45, CD68, CD86, CD169, CD206: Cluster of differentiation 11b, 45, 68, 86, 169, 206

CNS: central nervous system

CSF1R: colony stimulating factor 1 receptor

Cx3cr1: CX3C chemokine receptor 1, or CX3CL1 receptor

DAM: disease-associated microglial

DAMPs: damage-associated molecular patterns

DHA: docosahexaenoic acid

DIO: diet-induced obesity

DMV: dorsal motor nucleus of the vagus nerve

F4/80: cell surface glycoprotein

FIM: fat-induced microgliosis

GFP: green fluorescent protein

Glp-1: glucagon-like peptide 1

GLUT5 (or SLC2A5): glucose transporter 5

GPR120: G-protein coupled receptor 120

HFD: high-fat diet

Iba-1: ionized calcium-binding adapter molecule 1

IgG: Immunoglobulin G

IL-1β, IL6: interleukin 1β, 6

LPL: lipoprotein lipase

LPS: lipopolysaccharide

NPY: neuropeptide Y

NTS: nucleus of the solitary tract

NF-κB: nuclear factor-κB

ObRb: leptin receptor, isoform B

P2Y12: purinergic receptor

POMC: pro-opiomelanocortin

PUFA: polyunsaturated fatty acids

T2D: type 2 diabetes

TLR2, TLR4: Toll-like receptor 2, 4

TMEM119: transmembrane protein 119

TNFα: tumor necrosis factor α

integrin subunit alpha M

Keywords: Microglia, hypothalamus, energy homeostasis, food intake, eating disorders, lipids, inflammation.

Conflicts of interest: The authors declare no competing interests.

Abstract

Maintaining energy balance is essential for survival and health. This physiological function is controlled by the brain, which adapts food intake to energy needs. Indeed, the brain constantly receives a multitude of biological signals that are derived from digested foods, or that originate from the gastrointestinal tract, energy stores (liver and adipose tissues), and from other metabolically active organs (muscles). These signals, which include circulating nutrients, hormones, and neuronal inputs from the periphery, collectively provide information on the overall energy status of the body. In the brain, several neuronal populations can specifically detect these signals. Nutrient-sensing neurons are found in discrete brain areas and are highly enriched in the hypothalamus. In turn, specialized brain circuits coordinate homeostatic responses acting mainly on appetite, peripheral metabolism, activity and arousal. Accumulating evidence shows that hypothalamic microglial cells located at the vicinity of these circuits can influence the brain control of energy balance. However, microglial cells could have opposite effects on energy balance, i.e., homeostatic or detrimental, and the conditions for this shift are not totally understood yet. One hypothesis relies on the extent of microglial activation, and nutritional lipids can considerably change it.

Introduction

Microglia are resident immune cells of the central nervous system (CNS). Over a century ago, these cells were defined as the "third element" by Santiago Ramon y Cajal in reference to the two first elements already described, namely neurons and astrocytes. It was only in the 1920s that Pio del Rio Hortega specifically characterized microglial cells [1]. Arising from the embryonic yolk sac, microglia mostly colonize the brain parenchyma early during embryonic development, before the formation of the blood brain barrier [2] [3] [4]. This occurs around the fourth week of gestation in human [4] and around embryonic day 9 in mouse [5]. Additional sources and maturation programs could coexist [6]. Microglia represent a long-lived cell population that renew slowly and could persist the entire lifespan of the organism [7] [8].

Under physiological conditions, microglia are small cells that exhibit fine and ramified branches oriented radially from a small soma. Nevertheless, these cells are highly dynamic and can undergo back and forth morphological remodeling ranging from a "surveying" ramified state (small soma with abundant motile processes) to a "fully reactive" ameboid state (rounded swollen soma without branching) [9]. Although this morphological plasticity is thought to be linked to the reactive state [10] [11], this association remains controversial [12] [13]. In an attempt of classifying microglial reactive states, the concept of M1/M2 macrophage polarization was initially applied to microglia [14] [15] [16]. More recently, however, high-throughput approaches to study the remodeling of the whole microglial transcriptome under different pathological conditions failed to show evidence for microglia polarization along an M1–M2 axis [17]. This concept that did not consider microglia as long-lasting resident tissue macrophages highly adapted to the CNS environment [18] is now being abandoned (Paolicelli RC, Cell 2022, in press). Of note, the classification of M1/M2 peripheral macrophage polarization has now been also challenged [19] [20] [21].

Microglial cells ensure numerous functions in the CNS. Principally, these cells regulate brain development and contribute to brain homeostasis [22] [23] [24]. They can remove brain debris including synaptic components and dead cells according to their phagocytic ability [25] [26].

Microglia are also “sentinels” of the brain that constantly monitor the local environment, and they are the first line of defense in all CNS disturbances [27]. After acute injury or during a neuroinflammatory episode, microglia converge toward the site of brain region damage to restore the local physiological conditions. Short-term microglial reactivity is believed to be neuroprotective [28], while a prolonged microglial reaction can further increase tissue damage and negatively impact disease outcome [29] [30].

Recent advances in imaging techniques and genetic tools have enabled increased study of microglial cells. As a result, the concept of microglia heterogeneity has been established [31] [32] [6]. For visualization, the most widely used microglial markers are macrosialin (CD68), receptor-type tyrosine-protein phosphatase C (CD45), integrin subunit alpha M (CD11b), T-lymphocyte activation antigen (CD86), fractalkine receptor (CX3CR1), cell surface glycoprotein F4/80 and ionized calcium-binding adapter molecule 1 (IBA-1). Although resident CNS microglia have different origin with macrophages from the periphery, they share the above listed common markers. The recent identification of two specific microglial markers, namely the transmembrane protein 119 (TMEM119) and the purinergic receptor P2Y12, now allows discrimination between microglia and infiltrating CNS macrophages. According to their morphology and transcriptomic profiles, microglia may differ among and within brain regions [33] [6]. Transcriptomic analyses using single-cell RNA sequencing further revealed different molecular signatures between healthy microglia, also called homeostatic microglia, and disease-associated microglia. Age and sex are factors that might shape the microglial heterogeneity [34] [35] [36].

Beyond their role in injury, inflammation, and neurodegeneration, homeostatic microglia are involved in numerous physiological brain functions, including maturation of brain circuits during development, and modulation of synaptic transmission and plasticity in adults [37] [38] [13] [39] [40] [41] [42] [43] [44] [45] [46] [47] [48] [49] [50] [51]. In this way, the role of microglia in the neuronal circuits that control appetite and peripheral metabolism has recently emerged [52] [53] [54] [55]. However, the exact role of microglia in the regulation of food intake is not totally elucidated.

Actually, it is important to understand how microglia can sense food-derived cues, and how these cells could in turn drive feeding behavior because failure in these two distinct, interconnected, and complementary tasks, can reduce the ability of the organism to finely match food intake to energy needs, resulting in obesity, type 2 diabetes, or eating disorders. In particular, several recent works that will be presented below reveal that microglia located in the hypothalamus, a brain area involved in the regulation of energy balance, are strongly and specifically activated by nutritional lipids. Whether this process contributes to the macronutrient-dependent control of food intake or promotes neuronal dysfunction and accelerates the onset of obesity is still poorly understood. This review summarizes the current state of the field and proposes directions for future research.

Influence of nutrition on microglial activity

Early life programing. Neonatal overfeeding in rats conferred by a reduced litter size increases the number of Iba-1 positive microglia in the hypothalamic paraventricular nucleus [56] [57]. This constitutive microgliosis exacerbates central responses during immune challenges [56], and attenuates adaptive responses to short term high-fat diet (HFD) in adult [57]. Diet-induced maternal obesity in rats also causes constitutive microglial reactivity in the hippocampus of offspring at birth and exacerbates microglial reactivity after lipopolysaccharides (LPS) injection in adult [58]. This has been further observed in mice, in which maternal HFD alters transcriptional signature, morphology, and cell interaction of microglia in the hippocampus of offspring [59]. Maternal HFD also activates Iba-1 positive microglia in the fetal hypothalamus of macaques [60]. Although it is not known whether the cellular effect found in the brain of nonhuman primates has metabolic consequences, these data indicate that nutritional programming of microglia during critical neurodevelopmental periods is conserved across species. Moreover, these studies suggest that fetal and neonatal nutritional states prime responsiveness of hippocampic and hypothalamic microglia in adult. Such hypothalamic microgliosis induced by maternal nutrition seems to be sexually dimorphic. Actually, the microglial transcriptional priming induced by maternal HFD in mice is found in males only [59].

Moreover, after maternal HFD, cell interactions between microglia and astrocytes are distorted in males only [59]. On the other hand, neonatal overnutrition in rats does not cause sex dependent effects on microglia but increases reactivity of astrocytes in males only [61]. An additional point is that reduction in litter size in rats has no strong effect on Iba1-positive cells in the whole hypothalamus during early life, but considerably down-regulates Iba1 expression in adult brain [61], indicating that the nutritional programming of microglia during early life could have delayed molecular effects.

Metabolic state. Thorough inspection of Iba1-positive cells in mouse and rat brains according to the photic phase revealed a rhythmic pattern of microglial reactivity in the mediobasal hypothalamus [62]. Interestingly, this dynamic microglial reactivity is found in lean animals on standard diet, but not in diet-induced obese animals. Moreover, expression of clock genes in mouse microglial cells from lean animals follows a circadian rhythmicity, which is disturbed by chronic HFD [63] [64]. These data suggest that the microglial activity in the hypothalamus is coordinated conjointly by proper rhythmic oscillations of clock genes and by the periodic nutrient intake during normal physiology. Notably, postprandial activation of microglia is exacerbated by HFD [65]. Signaling pathways underlying microglial detection of daily changes in the metabolic state remain to be elucidated. Circulating nutrients may be elements of the microglial response. Indeed, a recent study suggests that changes in blood glucose are sensed by microglia. Specifically, insulin-induced hypoglycemia provokes microglial reactivity in the hypothalamus, which is characterized by an increased Iba1 immunostaining with enlarged Iba1-positive cells [66]. Although hypoglycemia is known to induce acidification of the extracellular environment that can activate microglia via acid sensing receptors, a direct sensing of glucose deprivation by microglial cells in this model remains possible. Interestingly, inhibition of hypoglycemia-induced microglial reactivity impairs the homeostatic counter-regulatory responses, demonstrating the contribution of microglia in the maintenance of energy homeostasis in lean animals. However, this study reveals a protective mechanism that is stimulated only during a supra-physiologic drop of glycaemia, and its role during

normal physiology is still unknown. Microglia are also activated during cachexia, a devastating syndrome characterized by anorexia and degradation of adipose tissues and muscles. Often associated with pancreatic and stomach cancer, cachexia is a particular metabolic state distinct from starvation [67] [68]. Starvation is a protein deficiency state often caused by lack of food, and can be reversed by refeeding. Cachexia is induced by systemic inflammation and is not reversed by refeeding. In a rodent model of cachexia, microglia accumulate rapidly and specifically in the hypothalamus, precisely in the arcuate nucleus and the median eminence, and not in the hippocampus nor the cortex [69]. Microglia depletion using PLX5622 worsens anorexia and muscle catabolism, demonstrating once again that microglial reactivity can be protective against a strong energy imbalance. Interestingly, data mining from human brain transcriptome databases found enrichment of microglia genes in anorexia nervosa (AN), a dramatic eating disorder, which is characterized by a profound energy imbalance with abnormally low body weight [70]. In the murine *anx*/*anx* model of anorexia, Iba1 immunoreactivity in the brain is increased as well as the number of Iba1-positive cells [71]. These studies identify microglia as an attractive target in anorexia nervosa research. However, it is still unknown whether microglia initiate, amplify or just respond to AN. Finally, in rats, chronic consumption of a ketogenic diet, a very low-carbohydrate and high-fat diet that mimics starvation, modifies microglial morphology, by increasing complexity and ramification of Iba1 positive cells [72]. *In vitro* studies demonstrate the high metabolic plasticity of microglia under starvation and reveal their versatile intracellular metabolic machinery [73]. Collectively, these studies show that microglia are able to sense negative energy state. However, it is hard to define whether specific morphological and functional microglial responses develop according to the origin and/or nature of the metabolic deficit.

Specific food products. Microglial activity can be significantly modified by daily consumption of specific food products and phytochemicals. There is a long list of micronutrients whose effects on microglia morphology and count have been demonstrated *in vivo* in experimental models. Most of

these compounds are contained in plant-based food and harbor health benefits. Among them we found gypenosides (triterpenoids extracted from *Gynostemma pentaphyllum*), magnolol (a polyphenol extracted from of *Magnolia officinalis*), catechins (polyphenols enriched in green tea) and berberine (an alkaloid extracted from Chinese herbs) [74] [75] [76] [77]. Fish oils enriched in omega-3 long chain polyunsaturated fatty acids (PUFA), such as docosahexaenoic acid (DHA), have also powerful effects on microglia morphology [78]. Importantly, low maternal intake of omega-3 PUFA can alter brain neurodevelopment via overactivation of microglial phagocytosis [79]. However, it remains to be established whether effects of these food-derived compounds on microglia are direct and/or associated with a better overall heath that reduces brain inflammation.

Metabolic sensors expressed by microglia. Microglia exhibit many molecular receptors and transporters that allow direct sensing of nutrients and metabolic hormones. Notably, microglia are sensitive to circulating leptin. This hormone acts on microglia *via* its ObRb receptor, which can in turn induce IL-1β release [80]. Leptin also modulates microglial reactivity and potentiates the microglial response to LPS [81]. Genetic ablation of the leptin receptor in CX3CR1-positive cells indicates that microglial leptin signaling is necessary for microglia appearance and behavior, and correct development of anorectic pro-opiomelanocortin (POMC) neurons, suggesting that microglia are part of the central effect of leptin [82]. Expression of this receptor in microglia can change according to the pathophysiological state. For instance, the leptin receptor is upregulated in spinal microglia during neuropathic pain and administration of leptin antagonist inhibits the development of microgliosis in the dorsal horn and brainstem [83]. The glucagon-like peptide 1 (Glp-1) receptor is also expressed in cultured microglia, and this receptor could be a modulator of inflammation in the central nervous system [84]. Interestingly, a 5-day treatment with exendin-4, a Glp-1 receptor agonist, in diet-induced obese mice reduces metabolic defects and microglial reactivity in the hypothalamus [85]. The mode of action of exendin-4 has not been established in this study, but pair-fed experiments suggest that exendin-4 can directly suppress diet-induced obesity (DIO)-associated

microglial reactivity. In addition, a treatment with exendin-4 given after an experimental stroke is neuroprotective in normal and aged T2D/obese mice, by promoting the expression of anti-inflammatory markers by microglia (Arg1, CD206, Ym1/2) [86]. Microglia can also sense sugars. Microglial expression of GLUT1 transporter, that facilitates the transport of glucose across the plasma membrane, might contribute to this capacity [87]. In addition, GLUT5, a fructose transporter, seems to be exclusively microglial in the brain [88]. Toll-like receptors (TLRs) are other sensors that recognize pathogen-associated molecular patterns (PAMPs) expressed by various infectious agents. Microglia express several of these receptors, including TLR2 and TLR4 [89] [90]. These receptors are sensitive to LPS that derive mainly from gut microbiota [91], but also to specific fatty acids [92] [93]. Lipid sensing by microglia is also ensured by the expression of GPR120, a receptor for unsaturated long-chain free fatty acids, and the lipoprotein lipase (LPL) [94] [95].

Role of microglia in the control of appetite

During the embryonic life, microglia seem to be crucial for proper neurodevelopment of brain circuits that control food intake. Elimination of microglial cells from fetal brain in mice with PLX5622, a CSF1R inhibitor, causes abnormal growth of pups and loss of anorectic POMC neurons [96]. This study shows long-term effects of embryonic microglia on energy balance and behavior. Similarly, genetic ablation of leptin receptor in CX3CR1-positive cells from the time of conception, modifies the morphology of microglia in the hypothalamus of adult mice, and decreases the number of POMC neurons and their projection fibers [82]. Moreover, these mice become hyperphagic and obese. In adult, depletion of microglia by PLX5622 does not affect food intake on standard diet but reduces it on HFD [97], suggesting that microglia in the adult brain are involved in the macronutrient-dependent control of food intake. Interestingly, depletion of microglia by PLX5622 rapidly alters the expression of neuropeptides that control food intake in the hypothalamus, showing a link between microglia and hypothalamic neurons [65]. The role of microglia in the regulation of appetite has been also studied after direct pharmacological activation of brain TLR2 receptors. Activation of microglial

TLR2 receptors causes anorexia and weight loss in rats [90]. Intracranial delivery of minocycline, which inhibits microglia reactivity, or liposome-encapsulated clodronate, which depletes microglia, mitigates TLR2-dependent anorexia and body weight loss, indicating that the microglial TLR2 pathway contributes to the control of food intake. In addition, TLR4 activation by LPS inhibits hypothalamic NPY neurons, and this response is blunted by minocydine [89], suggesting that microglia can directly modulate activity of neurons controlling food intake. Additionally, targeted manipulation of mitochondrial function in microglia alters food intake in HFD-fed mice only (not in lean mice), suggesting that the control of food intake probably varies according to the activation state of microglia [98].

How do microglia influence the neural circuits controlling feeding to cause the behavioral changes is not elucidated. Although several microglia-derived substances with direct neuroactive properties have been described [9], only few of them have been formally identified as mediators within brain circuits regulating food intake. For instance, microglia in the paraventricular nucleus of the hypothalamus express the brain-derived neurotrophic factor (BDNF), a factor that suppresses food intake [99]. BDNF deficiency in microglia leads to hyperphagia, obesity and insulin-resistance [99]. Moreover, targeted silencing of the nuclear factor-κB (NF-κB) in microglia, a transcription factor that triggers the inflammatory response, prevents weight gain in HFD-fed mice [97]. According to this study, pro-inflammatory factors might be secreted by microglia during diet-induced obesity, deregulating neurons that control energy homeostasis, stimulating weight gain. Indeed, microglia can release cytokines such as interleukin 1β (IL-1β), interleukin (IL6), and tumor necrosis factor α (TNFα), which alter neuronal activity [100] and energy metabolism [101] [102] [103] [104]. Likewise, TNFα from microglia alters neuronal firing rate of hypothalamic POMC neurons and increases body weight [62]. On the other hand, microglia are the major, if not the only, source of IL6 in the brain, and IL6 protects against obesity [105]. Finally, microglial metabolism also interferes with synaptic organization and leptin sensitivity in POMC neurons [98]. This raises the possibility that metabolic

end-products and/or ATP/AMP/adenosine might be elements of the microglia-to-neuron communication underlying the regulation of food intake.

Fat-induced microgliosis: a specific brain response to specific nutrients.

Early studies revealed that chronic HFD in adult provokes an important inflammatory reaction in the hypothalamus [106] that includes both a molecular aspect, such as the secretion of pro-inflammatory factors, and a cellular aspect, such as the activation of F4/80-positive immune cells [107]. Similarly, consumption of HFD can activate Iba1-expressing cells in the hypothalamus [108] [109]. Whatever the exact origin of these hypothalamic F4/80- and Iba1-positive cells that acutely react to nutritional lipids, namely resident microglia and/or newly recruited macrophages, it has become clear that the hypothalamus undergoes drastic cell remodeling in response to HFD that affects microglial cells, in a process that we propose to call fat-induced microgliosis (FIM) (Table 1). Increased Iba1-immunoreactivity has been evidenced in rodents after 1 and 3 day(s) on HFD, and also after 1, 4, 6, 8, 10, 12, 16, or 24 weeks on HFD [110] [111] [108] [85] [112] [95] [113] [114] [115] [62] [116] [117] [118] [98]. During long term HFD, Iba1-positive cells become large with highly ramified processes [108] [85] [62]. Such findings are variable according to the histopathological scoring and even not always detected [119] [120] [121]. Specific nutritional studies show that the nature of fat largely influences this process. Indeed, diets rich in saturated fatty acids have strong effects on microglia reactivity in the hypothalamus [113]. For instance, exposure to HFD supplemented in milk fat, which is highly enriched in saturated fatty acids, produces enlargement of the size of Iba1-positive cells in the mediobasal hypothalamus of mice [113]. This cannot be noticed after a week, but it becomes highly significant after a 4-week milk fat consumption. Interestingly, intragastric gavage with clarified milk fat can recapitulate this microglial process independently of the calorie intake, showing that the nature of ingested fat itself is a contributing factor. This applies, to a lesser degree, to coconut oil, but not to olive oil. Lard-based diets especially can accelerate this process [115]. On the other hand, C57BL/6 mice fed with a high-cholesterol high-salt Paigen diet for

8 weeks do not have major changes in hypothalamus and brain vessels, while *ApoE*$^{-/-}$ mice develop strong cerebrovascular inflammation and atherosclerosis, together with a general microglia activation in the whole brain under these nutritional conditions [122], supporting the idea that only some specific nutritional lipids are microglial activators. Moreover, combined overconsumption of fat and sugars amplifies FIM [112]. Interestingly, regular physical exercise can prevent or reduce FIM under chronic HFD [111] [123] [117], suggesting that intense utilization of substrates may limit the activation of microglia.

In addition to the physical enlargement of individual microglia following HFD, HFD-induced enhanced Iba1-immunoreactivity in the hypothalamus also includes an increase in the number of Iba1-positive cells [95] [85] [112] [113] [115] [62] [97] [124] [125] [116] (Table 1).

Consequences of FIM

Iba1 reactivity during long term HFD is associated with strong and specific deposition of IgG in hypothalamic microglia [108]. The exact mechanism underlying this diet-induced IgG deposition in the hypothalamus is not elucidated. The HFD-associated rise of blood IgG could have reached the brain and stimulated the phagocytic activity of microglia, or the HFD-induced activation of microglia could have boosted its scavenging function, independently of any IgG stimulation. Both pathways can promote IgG accumulation in the hypothalamus. In all cases, the pathophysiological impact of IgG accumulation in the brain during HFD is not known yet. In particular, it is unknown whether this process accelerates the HFD-induced alteration of hypothalamic control of energy balance or if this represents an adaptive response. Chronic HFD also increases levels of advanced glycation end products (AGEs) in the brain, which are biomarkers of aging and many neurodegenerative diseases. It has been proposed that microglia are responsible of the uptake clearance of AGEs secreted by hypothalamic neurons under HFD [112]. Indeed, disruption of this uptake reduces microgliosis and hypothalamic inflammation, indicating that AGEs clearance enhances FIM. These data support the idea that hypothalamic microglia protect the brain by removing age-related and obesity-induced

accumulation of damage-associated molecular patterns (DAMPs), but the functional counterpart on energy metabolism is not clear. Do reactive microglia aggravate diet-induced obesity or represent an adapted response that aims to maintain energy balance?

Several inflammatory markers are present in reactive hypothalamic microglia when consuming HFD for long term [113] [85] [112] and this is associated with cellular stress in neurons at the vicinity [113] [115]. Microglial depletion in mice brain using genetics and pharmacology, i.e., using diphtheria toxin receptor-mediated conditional and targeted cell ablation and Ki20227 (a CSF1R antagonist), clearly abolishes hypothalamic inflammation and neuronal stress induced by 3-day gavage with clarified milk fat [113]. In addition, microglial depletion in mice brain by PLX5622 enhances leptin signaling and limits food intake and body weight gain induced by 8-day gavage with clarified milk fat [113]. These data indicate that FIM induced by saturated fatty acid consumption can alter the control of energy homeostasis. In addition, after chronic HFD, microglia shift to a pathological state where they prematurely deplete synaptic numbers in the hippocampus, a brain region that serves a critical function in memory and cognition [110]. Inhibition of FIM by minocycline or by knock-down of *Cx3cr1*, prevents dendritic spine loss in hippocampic neurons and cognitive decline induced by HFD. ~~At the opposite,~~ Such manipulations of microglia do not influence cognitive performance in non-obese mice, suggesting that microglia play a detrimental role on cognition only when activated.

FIM could also have beneficial effects on energy balance. This possibility is supported by a recent study in mice carrying deficiency of lipoprotein lipase (LPL) in microglia [95]. These animals have altered basal microglial metabolism and immune responsiveness, showing firstly that microglial lipid sensing is crucial for brain functioning and homeostasis. Moreover, deletion of microglial LPL accelerates the onset of diet-induced obesity and increases the HFD-induced loss of POMC neurons, showing secondly that microglial lipid sensing is also important for brain control of energy homeostasis during HFD challenge. Strikingly, disruption of microglial LPL significantly attenuates

FIM, showing finally that metabolic alterations are aggravated when microglia are less reactive. These data suggest that microglia can have homeostatic function in energy balance.

Taken together, these studies show a dual role of FIM. This duality might depend on the progression of the pathological state with an early adaptive function during HFD exposure.

Specificities of FIM

Metabolic state. DIO models develop strong Iba1 immunoreactivity in the hypothalamus following HFD exposure. Interestingly, such Iba1 hyper-signal in the hypothalamus is not seen in genetically obese mice including *ob/ob* mice [85]. Similarly, HFD-induced IgG accumulation in the brain associated with microglial cells or IL-1β overexpression in microglia are not seen in the hypothalamus of *ob/ob* obese mice [108] [85]. These findings suggest that dietary lipids consumption but not obesity or adiposity *per se* is a causal element in this hypothalamic response. Remarkably, Iba1 immunoreactivity in the hypothalamus is low in lean mice and variable according to the prandial state, while permanently elevated in DIO mice [62] [65].

Localization. Expansion and enlargement of Iba1-positive cells during HFD is most evident in the hypothalamus [124] [125]. This response seems to be restricted to the mediobasal part of hypothalamus, in particular in the arcuate nucleus [85] [126], and more marked in the caudal part of this nucleus [127]. As well, infiltration of macrophages in response to HFD is found in the hypothalamus, but not in the cortex [126]. Similarly, the increased F4/80 immunoreactivity induced by chronic HFD is detected in the arcuate nucleus of the hypothalamus, but not in frontal, parietal, occipital and cerebellar cortices [107]. FIM has been also observed in the hippocampus [110] [128] [95] [120], the cerebellum [121], and in the brainstem. In the latter, FIM occurs specifically in the nucleus of the solitary tract (NTS), which is the first relay for visceral (vagal) and taste afferents toward the brain, and in the dorsal motor nucleus of the vagus (DMV), which is the origin of (vagal) gastrointestinal-projecting motor neurons [129] [130]. Moreover, in the peripheral nervous system,

FIM has been detected in nodose ganglia, sources of the vagal afferents [116] [131] [118]. These data highlight the relative spatial specificity of FIM that affects mainly nervous structures controlling feeding behavior, as it has been observed for HFD-induced astrogliosis [132].

Cell expansion. HFD increases the number of Iba1-positive cells in the hypothalamic parenchyma of rodents [85] [113] [115] [62] [97] [124] [125] [126]. However, it is important to note that Iba1 immunolabeling can detect both resident microglia and infiltrated macrophages. Using a series of specific microglial markers including TMEM119 and P2Y12, it has been shown that the number of resident microglial cells in the medio-basal hypothalamus is not increased after 4-week HFD [97]. Conversely, HFD-fed mice had a significant rise in CD169-positive cells in the hypothalamus. CD169 is a marker of monocyte-derived cells, but not brain microglia. The increase in CD169-positive cells is not seen after 1 week on HFD but becomes significant at 4 and 8 weeks on HFD. Transplantation of GFP-tagged bone marrow-derived monocytes in mice allowed tracking of circulating myeloid cells, demonstrating their infiltration in the hypothalamus during HFD [97]. This study, which is in line with others [133] [126], further indicates that recruited cells can activate resident microglia. Analysis of chimeric animals reveals also that local perivascular and meningeal macrophages, which are $CD68^{pos}$ $TMEM119^{neg}$ GFP^{neg} cells, can also be recruited in the hypothalamus during HFD [97]. Complementary analyses using BrdU staining and/or Ki67 proliferative marker did not report strong induction of proliferation of Iba1-positive cells in the brain during HFD [85] [134] [97]. All these studies suggest that the increase in the overall number of microglial cells in the hypothalamus following HFD does not involve proliferation of resident cells but may be due to a large infiltration of circulating cells and recruitment of distant brain cells.

However, the origin of the HFD-induced increase in the number of Iba1-positive cells in the hypothalamus is still matter of debate. Indeed, recent works do not reveal any infiltration of monocytes in the hypothalamus under long-term HFD [135] [125]. The reasons for the discrepancies between these studies are unclear, but might include technical issues (i.e. impact of irradiation for

cell transplantation studies on the integrity of the blood brain barrier), differences in models (i.e. nature of diets, metabolic state), and time point of analysis, as inflammatory diseases are progressive with episodic flare-ups and life span of myeloid cells varies according to their differentiation state and activity [136].

In addition, some studies reported that some Iba1-positive cells within the hypothalamic parenchyma could proliferate in response to long term HFD [124] [125]. Such seemingly conflicting result may account for the expansion of resident hypothalamic perivascular and parenchymal macrophages, as already reported after 2, 4 or 20 weeks of HFD [137], and (perhaps) also of newly infiltrated macrophages that can undergo rapid proliferation *in situ*, as it happens in the periphery during chronic HFD [138] [139]. Therefore, additional studies are needed to better understand the cellular origin of the hypothalamic Iba-1 positive cell pool that expanses under HFD. Tracking these cells requires invariable cell phenotype and stable expression of identity markers, which might not be the case over the course of the HFD feeding.

Sex specificity. As mentioned above, depletion of microglia during neurodevelopment causes loss of POMC neurons, revealing the importance of microglia in brain feeding circuits [96]. Interestingly, this treatment also promotes hyperactivity and anxiety, but this behavior develops in females only [96]. Moreover, cell interactions between microglia and astrocytes are distorted in males but not in females after maternal HFD [59]. These observations support the concept that early programming of microglia has functional and behavioral outcomes with substantial sexual differences [140] [141].

Interestingly, male microglia are more likely to express pro-inflammatory genes, compared to female microglia [34] [140] [141]. The sexual dimorphism of microglia could be explained by the perinatal exposure to sex steroid hormones [142] [34]. Anti-inflammatory effects of estrogens on microglia still persist during adult life [143] [144]. At the molecular level, the estrogen receptor alpha (ERα) contributes to the determination of microglia immunocompetence during neurodevelopmental

stages and during adult life. The corollary is that the loss of estrogens at menopause promotes phenotypic changes in female microglia with aging [144]. These studies indicate that adult microglia show a sexual dimorphism, which may explain differences in prevalence of some neurological disorders [145] [34].

Under normal nutritional condition, basal morphology of Iba1-positive cells in the hypothalamus differs according to the sex [61]. Dynamic morphological reactivity of microglia in response to HFD is also sex-dependent: hypothalamic FIM has been observed in the hypothalamus of male but not female mice [126]. Unlike male microglia, microglia from HFD-fed female mice showed no increase in pro-inflammatory *Il1b* and *Ikbkb* gene expression, indicating that female mice are protected from HFD-induced hypothalamic microglial activation [146]. Sex-specificity in microglial CX3CR1 signaling seems to be a major determinant of FIM [146]. In particular, female CX3CR1 knockout mice develop a "male-like" FIM in the hypothalamus and an increased susceptibility to diet-induced obesity.

Species specificity. Contrary to C57Bl/6J mice, Wsb/EiJ mice do not develop intense FIM in the arcuate nucleus of hypothalamus [114]. As Wsb/EiJ mice are resistant to DIO, it has been proposed that these mice have more efficient homeostatic systems that manage lipid overload, use and storage, in relation with a higher central sensitivity to nutritional and metabolic cues. However, it is also still unknown whether increased FIM in C57Bl/6J mice reveals a pro-inflammatory response that accelerates the onset of obesity, or a defensive homeostatic response intending to restore the rupture in energy balance, which is not necessary in Wsb/EiJ mice.

Pathophysiological specificity. Phagocytosis, chemotaxis, immune cell recruitment and phenotypic changes of microglia are not restricted to high-fat feeding. Many neuroinflammatory states, including ageing, Alzheimer disease (AD), amyotrophic lateral sclerosis, Parkinson disease, X-linked adrenoleukodystrophy and multiple sclerosis share these morphological and functional

microglial changes [147] [148]. Moreover, a common transcriptomic program in activated microglia, named disease-associated microglial (DAM) signature, has been found in neuroinflammatory diseases and ageing [147] [149]. Nevertheless, FIM appears as a singular, and maybe even paradoxical, biological response that associates extension and ramification of the microglial processes along with inflammatory activation (Table 1). Whether FIM adopts a DAM signature needs to be determined.

Future directions

Current data show that FIM occurs in specific brain areas that control food intake and maintain energy homeostasis. What remains to be determined is whether the distinct microglia behavior in these areas is due to a particular microenvironment exposing microglia to specific signals, or whether microglia located in these areas constitute a singular subpopulation with specific molecular receptors that provide them with unique reactiveness, or both. Comparative studies using targeted molecular analyses, such as single-cell RNA sequencing, on distinct pools of microglia from brain areas that undergo FIM or not would help to address this issue. Spatial heterogeneity of microglia during homeostasis in the adult healthy brain is still matter of debate [150]. Nevertheless, microglial heterogeneity has been demonstrated in the context of Alzheimer disease by RNA-seq [151]. The analysis reveals that only one specific microglial subset is altered in Alzheimer disease, indicating the existence of specific microglial responses in the diseased brain.

Mechanistic studies in healthy animals fed a standard diet reveal that resident microglia modulate the activity of neurons involved in the control of energy homeostasis and contribute to the regulation of food intake. However, the role of microglia when activated is still debated. As any other inflammatory responses that operate during severe disturbances of homeostasis, FIM could have different physiological purpose and pathological consequences according to the intensity and the duration of the stressor [152]. Thus, whether FIM is a deleterious response that promotes metabolic imbalance or rather an adaptive response that normalizes energy balance is still unsolved. Maybe the

answer is twofold depending first on the duration of the HFD exposure and the possible repetition of the HFD episodes, and second on the age and the metabolic state of the animals. Again, comparative studies at different time-point under HFD would be helpful to settle the matter.

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JS, PA, EA, EB, SBF, MCM, TH, FL, EN, CR, SS, AV, DT, and AB wrote the manuscript. JS, DT and AB prepared the plan of the article.

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Table 1. Characteristics of fat-induced microgliosis (FIM) in the hypothalamus.

Ref	Animals	HFD		FIM				
		Composition	Duration	Brain area	Cell marker	Cell number	Cell size	Cell morphology
Yi 2017 [44]	Male C57BL/6JRj mice	58% fat (coconut oil, soybean oil)	4 months	ARC	Iba1	↗	NA	↗ processes
Gao 2014 [61]	6-week old CX3CR1-eGFP mice	NA	6 weeks	ARC	Iba1, Cx3cr1-eGFP	↗	NA	NA
	C57BL/6 mice		10 weeks		Iba1, CD68	↗	NA	↗ ramification
	$Lep^{ob/ob}$ mice		2 weeks		Iba1, CD68	↗	NA	↗ ramification
	$Lepr^{db/db}$ mice		2 weeks		Iba1, CD68	↗	NA	= ramification
Cansell 2021 [70]	8-week old male C57BL/6J mice	40.9% fat (lard)	• 3 hours • 6 hours	ARC	Iba1	NA	NA	• ↗ cell capacitance • ↗ soma size
Valdearcos 2017 [70]	12-16-week old male C57BL/6 mice	42% fat (milk fat) Or 60% fat (lard, soybean oil)	4 weeks	ME	• Iba1 • P2Y12 • Tmem119	• ↗ • ↘ • ↘	NA	NA
				ARC	• Iba1 • P2Y12 • Tmem119	• ↗ • ↘ • ↘	NA	NA
				VMH	• Iba1 • P2Y12 • Tmem119	• = • = • =	NA	NA
	12-16-week old male Cx3cr1-GFP mice		• 1 week • 4 weeks • 8 weeks	MBH	• Cx3cr1+/P2Y12- • Cx3cr1+/Tmem119 • Cx3cr1+/P2Y12- • Cx3cr1+/Tmem119 • Cx3cr1+/P2Y12- • Cx3cr1+/Tmem119	• = • = • ↗ • ↗ • ↗ • ↗	NA	NA
Kim 2019 [72]	10-week old male $Cx3cr1^{CreE}$/R26 tdTomato mice	45% fat (lard, soybean oil)	• 3 days • 7 days • 8 weeks	ARC	Cx3cr1-tdTomato	• = • ↗ • ↗	• ↗ • ↗ • ↗	NA
	10-week old male $Ucp2^{MGKO}$ / tdTomato mice		• 3 days • 7 days • 8 weeks			• = • = • =	• = • ↗ • ↗	NA
Yi 2012 [74]	Male C57BL/6 mice	58% fat (coconut oil, soybean oil)	16 weeks	ARC	Iba1	NA	↗ soma	↗ ramification
Berkseth 2014 [75]	Male C57BL/6 POMC-tau-green fluorescent protein (GFP) mice	60% fat (lard, soybean oil)	20 weeks	ARC	Iba1	=	NA	• ↗ activation score • ↗ cell bodies • ↗ ramification
Klein 2019 [77]	6-week old female C57BL/6J mice	60% fat (lard, soybean oil)	12 weeks	ARC	Iba1	↗	NA	Ramified microglia + ameboid microglia

Gao 2017 (78]	10-week old WT mice	58% fat + sucrose	4 weeks	ARC	Iba1	↗	↗	NA
		61.9% fat + starch				↗	↗	
		78.7% fat				=	↗	
		92.8% fat				=	=	
Valdearcos 2014 [79]	10-week old C57BL/6 mice	42% fat (milk fat)	• 1 week • 4 weeks • 16 weeks	ARC	Iba1	• = • ↗ • ↗	• = • ↗ • ↗	• NA • NA • NA
Terrien 2019 [80]	12-week old male C57BL/6J mice	45% fat (lard, soybean oil)	• 3 days • 8 weeks	ARC	Iba1	• = • =	• ↗ • ↗	NA
	12-week old male WSB/EiJ mice		• 3 days • 8 weeks			• = • =	• ↗ • ↘	
Thaler 2012 [81]	Male Long-Evans rats	60% fat (lard, soybean oil)	• 1 day • 3 days • 7 days • 14 days	ARC	Iba1	• ↗ • ↗ • ↗ • ↗	• ↗ • ↗ • ↗ • ↗	• NA • NA • ameboid microglia • NA
Waise 2015 [82]	6-week old male C57BL/6J mice	60% fat (lard, soybean oil)	1 day	ARC	Iba1	↗	↗	• Rounded • ↗ ramification
Yin 2018 [83]	12-week old male C57BL/6J mice	45% fat (lard, soybean oil)	• 3 months • 6 months • 9 months	HT	Iba1	• = • = • ↗	NA	NA
Naznin 2015 [84]	6-week-old male C57BL/6J mice	60% fat (lard, soybean oil)	12 weeks	HT	Iba1, CD11b, CD86	↗	NA	• Rounded • ↗ ramification
Harrison 2019 [85]	Male C57BL/6JRj mice	58% fat (coconut oil, soybean oil)	22 weeks	ARC	Iba1	=	NA	• =
Daly 2020 [86]	8-week old male and female C57BL/6J mice	45% fat (lard, soybean oil)	14 weeks	HT	Iba1	NA	NA	• ↘ cell complexity (only in male) • = processes length • = branch numbers • = cell shape
André 2017 [90]	7-week old male C57BL/6J mice	60% fat (lard, soybean oil)	3 weeks	ARC	Iba1	↗	↗ soma	NA
Baufeld 2016 [91]	14/17-week old male C57BL/6J mice	60% fat (lard, soybean oil)	• 3 days • 4 weeks • 8 weeks	HT	Iba1	• = • = • ↗	NA	NA
	C57BL/6J Actin-GFP bone marrow chimeric mice		20 weeks			↗	NA	NA

ARC: Arcuate Nucleus; CD: Chow Diet; Cx3cr1: CX3C chemokine receptor 1; DIO: Diet-Induced Obesity; eGFP: enhanced Green Fluorescent Protein; HCHF: High Carbohydrate High Fat; HFD: High-Fat Diet; HT: Hypothalamus; Iba1: Ionized calcium binding adaptor molecule 1; IHC: Immunohistochemistry; Kcal: Kilo calorie; LCHF: Low Carbohydrate High Fat; LFLS: Low Fat Low Sugar; MBH: Medio-Basal Hypothalamus; NA: Not Analyzed; P2Y12: Purinergic Receptor P2Y12; SC: Standard Chow; SD: Standard Diet; SFAs: Short Fatty Acids; Tmem119: Transmembrane Protein 119; VMH: Ventromedial nucleus or the Hypothalamus; WT: Wild Type