Hypothalamic innate immune reaction in obesity

Stefanie Kälin, Frank L. Heppner, Ingo Bechmann, Marco Prinz, Matthias H. Tschöp and Chun-Xia Yi

Abstract | Findings from rodent and human studies show that the presence of inflammatory factors is positively correlated with obesity and the metabolic syndrome. Obesity-associated inflammatory responses take place not only in the periphery but also in the brain. The hypothalamus contains a range of resident glial cells including microglia, macrophages and astrocytes, which are embedded in highly heterogenic groups of neurons that control metabolic homeostasis. This complex neural–glia network can receive information directly from blood-borne factors, positioning it as a metabolic sensor. Following hypercaloric challenge, mediobasal hypothalamic microglia and astrocytes enter a reactive state, which persists during diet-induced obesity. In established mouse models of diet-induced obesity, the hypothalamic vasculature displays angiogenic alterations. Moreover, proopiomelanocortin neurons, which regulate food intake and energy expenditure, are impaired in the arcuate nucleus, where there is an increase in local inflammatory signals. The sum total of these events is a hypothalamic innate immune reactivity, which includes temporal and spatial changes to each cell population. Although the exact role of each participant of the neural–glial–vascular network is still under exploration, therapeutic targets for treating obesity should probably be linked to individual cell types and their specific signalling pathways to address each dysfunction with cell-selective compounds.

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Introduction

Over the past three decades, levels of obesity have reached epidemic proportions. Tremendous efforts have been made by scientists to search for antiobesity treatments to combat this epidemic, as well as diseases associated with obesity. Malfunction of the central nervous system (CNS) network that controls energy intake and expenditure is the major mechanism for the development of obesity.[1–3] In individuals with obesity, one of the hallmarks of the pathological changes in peripheral organs and tissues is insulin resistance driven by proinflammatory responses involving macrophages and adipocytes.[4,5] In the past decade, reactive changes in the hypothalamus have come to the attention of researchers, as they have been shown to be triggers of leptin and insulin resistance.[6,7] This Review will discuss the innate immune response in the hypothalamic neuron–glial circuit to obesity and associated metabolic disorders and propose corresponding strategies for treating obesity.

The hypothalamus

The hypothalamus is one of the most heterogeneous structures in the CNS, containing highly condensed populations of neurons. These neurons are responsible for a variety of neuroendocrine and autonomic functions, including controlling feeding, reproduction and defensive responses.[8,9] Situated in the mediobasal hypothalamus, the arcuate nucleus (ARC) contains orexigenic agouti-related peptide (AgRP)–neuropeptide Y (NPY) neurons and anorxegenic proopiomelanocortin (POMC) neurons. These neurons express receptors for key metabolic regulatory hormones such as insulin, leptin, ghrelin, glucocorticoids, estrogen, thyroid hormone and glucagon-like peptide 1 (GLP-1).[9] Located close to the ARC, the ventromedial hypothalamus is an area that couples metabolic sensing to the reproductive control circuit[10] by expressing not only insulin, leptin and estrogen receptors, but also steroidogenic factor 1[11] and brain-derived neurotrophic factor.[12] Metabolic sensing signals from the ARC are conveyed to second-order hypothalamic regions, including the paraventricular nucleus. The paraventricular nucleus hosts neuroendocrine neurons that control the stress response, as well as the pre-autonomic sympathetic and parasympathetic neurons that can balance the peripheral energy production and expenditure by controlling glucose and lipid metabolism.[13–15] These neurons are embedded within the glial population, which includes microglia, astrocytes and tanycytes (Figure 1, Box 1). Furthermore, the mediobasal hypothalamus is positioned next to the median eminence, which is one of the circumventricular organs, and so this area is highly sensitive to blood-borne factors.[16]

As the hypothalamus monitors metabolic challenges, it is not surprising that it is the first location where a highly reactive response can be detected when animals are exposed to a hypercaloric environment.[6,7,17,18] The reactive response is characterized by glial reactivity, secretion of cytokines and increased levels of intracellular

Institute for Diabetes and Obesity, Helmholtz Centre for Health and Environment & Technische Universität München, 85748, Munich, Germany (**S.K.**, **M.H.T.**). Department of Neuropathology, Charité, Universitätsmedizin Berlin, 10117 Berlin, Germany (**F.L.H.**). Institute of Anatomy, University of Leipzig, Liebigstr. 13, 04103 Leipzig, Germany (**I.B.**). Institute of Neuropathology, University of Freiburg, Breisacher Str. 64, D-79106 Freiburg, Germany (**M.P.**). Department of Endocrinology and Metabolism, Academic Medical Center, University of Amsterdam, Meibergdreef 9, 1105 AZ, Amsterdam, Netherlands (**C.-X.Y.**).

Correspondence to: M.H.T. matthias.tschoep@helmholtz-muenchen.de

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The authors declare no competing interests.

Key points

- Hypothalamic metabolic sensing requires an interactive network of neurons, glial cells and vasculature to enable appropriate integration of complex metabolic feedback signals
- Exposure to hypercaloric environments induces inflammatory-like responses not only in peripheral tissues but also in the central nervous system, especially in the hypothalamus
- The term 'hypothalamic inflammation' is being used more and more frequently to describe a complex of hypothalamic processes that occurs in response to hypercaloric diets
- The phenomenon of hypothalamic inflammation in obesity is more similar to an innate immune reaction than to conventionally defined inflammation
- To address the hypothalamic innate immune reaction therapeutically, cell-specific strategies should be developed that enable prevention of the adverse effects of systemic treatment approaches

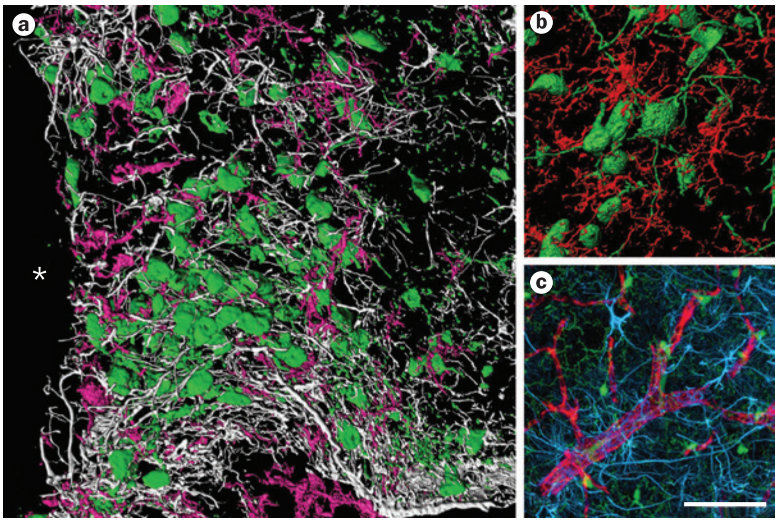

Figure 1 | The cytoarchitecture of hypothalamic NPY and POMC neurons, microglia, astrocytes and vasculature. **a** | A 3D reconstruction of NPY neurons (green), AIF-1-ir microglia (pink) and GFAP-ir astrocytes (white) in the arcuate nucleus. **b** | A 3D reconstruction of POMC neuron (green) and microglia (AIF-1-ir, in red) in the arcuate nucleus. **c** | Blood vessel (red) with microglia/perivascular macrophages (from CX3Cr1-GFP, green) and GFAP-ir astrocytes (blue) in the mediobasal hypothalamus. Asterisks denote location of the third ventricle. Scale bar: 35 μm in a, 50 μm in b and 70 μm in c. Abbreviations: AIF-1, allograft inflammatory factor 1; GFP, green fluorescent protein; GFAP-ir, immunofluorescent staining of glial fibrillary acidic protein; NPY, neuropeptide Y; POMC, proopiomelanocortin.

inflammatory signals. In rodent models of long-term diet-induced obesity (DIO), glial activation is sustained and the vasculature undergoes angiogenesis.[19] In addition, POMC neurons, which inhibit food intake and stimulate energy expenditure, show reduced functionality.[18,20,21]

This hypercaloric-induced activation of the hypothalamic neural–glial network is different from the alterations that occur in canonical neuroinflammatory diseases. These diseases are induced by reactivity to neural self-antigens, bacteria, viruses and other pathogens and typically involve adaptive immune cells such as B cells and T cells. The hypercaloric-induced activation of the hypothalamic neural–glial network resembles conditions in which the innate immune system is the major disease-promoting factor, as seen in degenerative CNS diseases such as Alzheimer disease.[22,23] How these pathophysiological changes in the hypothalamic neural–glial network are connected to CNS control of energy metabolism, and whether these changes are the cause or the consequences of obesity, is currently an area of intense study. Unravelling the specific role of each cell population in this network and/or specific cell signalling pathways that are responsible for the development of obesity will help in the search for the most effective therapeutic treatments.

Hypothalamic microglia and inflammation

Microglia and macrophage heterogeneity

Microglia in the CNS originate from c-Kit^{+} erythromyeloid progenitors that are derived from the extra-embryonic yolk sac that colonizes the developing brain.[24–27] These cells form a stable and autonomous population, which is not connected to the myeloid cell population derived from bone marrow that is normally found in the circulation, and which is only able to engraft the unchallenged CNS under experimental conditions.[25,28–30] Therefore, it can be assumed that postnatal microglia are maintained independently of circulating monocytes throughout life.[24] In the healthy CNS environment, microglia comprise ~10% of the cells in the brain and are engaged in immune surveillance, as well as homeostatic regulation and remodelling of neuronal activity.[31] Microglia are considered to be the primary sensors of pathological changes in the CNS.[32] Furthermore, these cells are the first to respond to injury in the CNS by transforming from their surveillance state to an activated form characterized by migration, proliferation and active communication with neighbouring neurons and non-neuronal CNS cells.[33] The latter is achieved by the release of various cytokines and chemokines.

As in other brain regions, microglia in the hypothalamus display several different phenotypes. Such heterogeneity might reflect the highly specialized and adaptive nature of microglia under region-specific conditions, for example, the presence of certain neurotransmitters. The most complex area in the hypothalamus is the median eminence, which contains a highly fenestrated vascular endothelium that lacks a blood–brain barrier. Together with the adjacent ARC, the median eminence forms a hot spot in the hypothalamus and functions as the 'brain window' for intensive communication between the mediobasal hypothalamus and the general circulation. The median eminence develops during an early postnatal stage (at about day 14), which is much later than the first appearance of mature microglia in the CNS, and is accompanied by a rapid intrusion of nerve terminals of neurosecretory neurons.[34,35]

A similar process is the pruning of neurosecretory terminals by microglia in the pituitary gland.[36] Execution of this pruning requires microglia that have proliferated from pre-existing microglia in the ARC, or myeloid cells that have been recruited from bone-marrow-derived circulating precursors. In fluorescent fractalkine receptor CX3CR1–eGFP mice,[37] the median eminence internal zone, an extension of the ARC that contains a few NPY (Figure 2a,b) and POMC neurons as well as astrocytes and tanycytes (Figure 2c), is populated with microglia that are CX3CR1+ and that express the myeloid marker allograft inflammatory factor 1 (AIF-1). The external zone of the median eminence, however, contains highly ramified microglia and non-ramified, rod-like cells that morphologically resemble macrophages (Figure 2d). Moreover, not all ramified CX3CR1+ cells in the external zone display AIF-1 immunoreactivity (Figure 2d). The external zone of the median eminence is not considered to be part of the blood–brain barrier, as microglia in this region can be exposed to, and subsequently become increasingly activated by, circulating factors, which drives development of a macrophage-like morphology. Several questions remain concerning this macrophage-like population. Firstly, do they function as guardians of a leaking blood–brain barrier, preventing the invasion of pathogenic factors from the median eminence into the ARC? Secondly, do they respond to metabolic challenges such as overloading nutrients? Thirdly, do they have a short lifespan and undergo a turnover rate similar to that of peripheral monocytes?

In addition to residing within the median eminence, hypothalamic CX3CR1+ cells are tightly attached to the blood vessel walls. These elongated cells can be identified by green fluorescent protein (GFP) in CX3CR1-eGFP mice and by the lack of AIF-1 (Figure 2e). Perivascular macrophages express the surface markers CD163 and CD206,[38] but concomitantly lack AIF-1 immunoreactivity (M. Prinz, unpublished work). Perivascular macrophages are thought to be more active than microglia within the parenchyma, which might facilitate the communication between injured CNS parenchyma and circulating immune cells.[39] In animal models of Alzheimer disease, the depletion of perivascular macrophages increased the severity of cerebral amyloid angiopathy, and stimulation of their turnover rate promoted the clearance of cerebral amyloid angiopathy.[38] However, in an animal model of multiple sclerosis, clustering of perivascular macrophages seemed to be required for neuronal axonal damage induced by experimental autoimmune encephalomyelitis.[40] Thus, the exact function of hypothalamic perivascular macrophages needs to be elucidated.

Box 1 | Figure preparation

All pictures were obtained from mouse brain samples. Brain tissue was prepared by paraformaldehyde perfusion or immersion fixation and cut on a cryostat into 30 μm sections. Microglia (except for those from the CX3CR1-GFP mouse) are visualized by immunohistochemical or immunofluorescent staining of allograft inflammatory factor 1. Astrocytes (except for those from the hGFAP-GFP mouse) are visualized by immunofluorescent staining of glial fibrillary acidic protein (GFAP-ir). All vessels are visualized by labelling with albumin conjugated with Alexa Fluor® (Thermo Fisher Scientific, Waltham, MA USA) 555 dye infusion via tail vein for 1 min. NPY and POMC neurons are visualized by GFP from NPY-GFP and POMC-GFP transgenic mice.

Abbreviations: GFAP-ir, immunofluorescent staining of glial fibrillary acidic protein; GFP, green fluorescent protein; NPY, neuropeptide Y; POMC, proopiomelanocortin.

Microglial phagocytosis in the hypothalamus

In general, microglia are responsible for cleaning up invading pathogens from the periphery as well as the debris produced by residential cells in the developmental and the adult brain. An increased phagocytic capacity is considered to be beneficial for eliminating debris and inducing an anti-inflammatory response to maintain homeostasis in the local microenvironment.[41] The classic phagocytic process includes recognition, engulfment and digestion of the targets. The target-recognition process can be started through the opsonisation of the pathogens with IgG, which triggers microglia to phagocytize the target via Fc receptors,[42–44] a process that involves complement receptor 1.[45] This IgG–Fc receptor-mediated phagocytosis has been used to deliver antibodies against the Aβ peptide and successfully reduced the plaque burden in a mouse model of Alzheimer disease.[46] However, the detailed mechanisms of these Aβ-targeted immunotherapeutic approaches, including the role of the microglia, are still not understood.[47] In naive wild-type C57BL/6 mice, microglial deposition of IgG has been observed in many different brain areas.[48] Interestingly, even in standard chow-fed conditions, levels of deposited IgG are raised in a very well-defined region within the hypothalamic ARC and median eminence and increased further after the mice were fed a high-fat diet (HFD).[49] The trigger for IgG over-deposition in the HFD condition could be a result of a leaky blood–brain barrier in this area. This leaky state would enable macromolecules, including pathogenic factors, to enter the CNS from the general circulation and lead to microglial activation.

An alternative explanation for the increased deposition of IgG in microglia is that the 'brain window' of the ARC and median eminence produces more debris than other brain regions, as these areas contain highly activated neurons that monitor the peripheral metabolic state. Microglial phagocytosis could also be regulated locally by neuropeptides in the hypothalamus. Orexigenic peptide NPY produced in the ARC is able to inhibit IL-1β-induced microglial phagocytosis.[50] The NPY-regulated microglial phagocytic activity, therefore, could be an essential cooperating step for the neural–glial network in the adaptation to metabolic challenges. Ultimately, microglial phagocytosis could be compromised site-specifically by an excessive supply of neuropeptides, nutrients, macromolecules or debris. This compromised function has been shown for Alzheimer disease, where dysfunction of microglia (including impairment of phagocytosis) seems to result from the deposition of the pathological Aβ protein, thus possibly exacerbating the disease course.[51]

Another important role of microglial phagocytosis is the selective elimination of synapses and/or neurons during CNS development and in the brain during the first 3 weeks of life, a process termed pruning.[52,53] In mice, deficits in developmental microglia pruning effect functional brain connectivity and result in autism-like behaviour.[54] Interestingly, a developmental deficiency of neurite growth in the hypothalamus can be found in

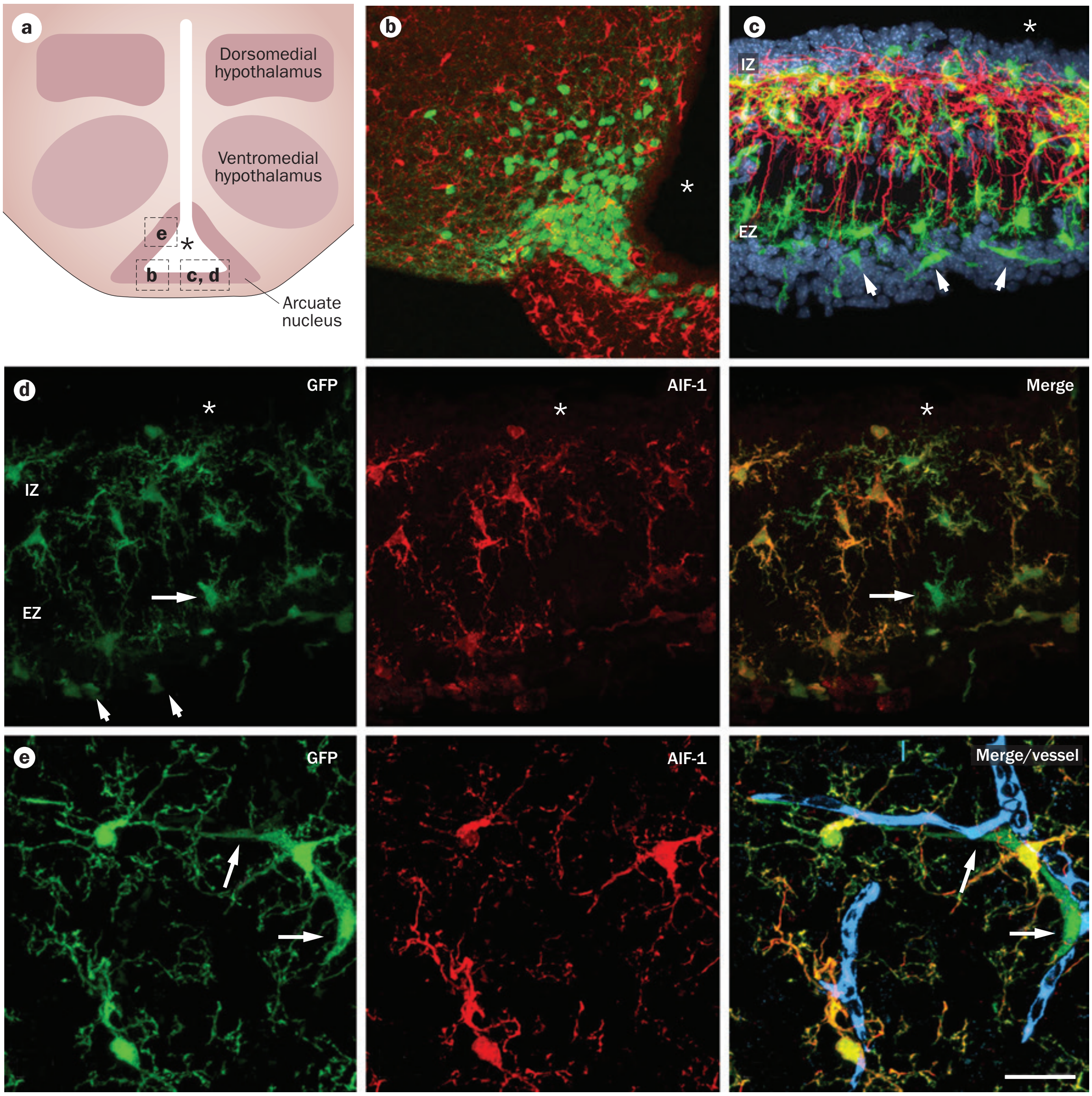

Figure 2 | The diversity of hypothalamic microglia and macrophages visualized in CX3Cr1-GFP mice. **a** | Microglia and macrophages in different locations of the mediobasal hypothalamus. **b** | NPY neurons (from NPY-GFP mice, in green) extend into the internal zone of the ME, while microglia (by AIF-1-ir in red) are densely distributed throughout the entire ME. **c** | The cytoarchitecture of microglia and macrophages (in green), astrocytes and tanycytes (GFAP-ir, in red) in the IZ and EZ of the ME (indicated by DAPI in blue). The white arrows point to macrophage-like cells in the EZ of the ME. **d** | GFP$^+$ (green) or AIF-1-ir$^+$ (red) microglia and macrophages in the ME, white arrow points to a CX3CR1$^+$ cell without AIF-1-ir in the EZ of the ME. **e** | Two perivascular GFP$^+$CX3CR1$^+$ cells (indicated by white arrows) tightly apposed to blood vessels (in blue) in the mediobasal hypothalamus. 'Merge', shown in yellow, indicates microglia and macrophages that are positive for GFP and AIF-ir. Asterisks denote location of the third ventricle. Scale bar: 70 μm in b, 50 μm in c and d and 20 μm in e. Abbreviations: AIF-1, allograft inflammatory factor 1; EZ, external zone; GFP, green fluorescent protein; GFAP-ir, immunofluorescent staining of glial fibrillary acidic protein; IZ, internal zone; ME, median eminence; NPY, neuropeptide Y.

monogenic obese (*ob/ob*) mice that lack leptin.[55] As it is known that leptin can regulate microglial phagocytosis,[56] it is conceivable that a malfunction of microglial pruning caused by leptin deficiency is involved in the growth deficits of hypothalamic neurites.

Determination of microglial phagocytic capacity mostly relies on a qualitative observation of microglial engulfment.[57] As phagocytosis is a very dynamic process, it would be ideal to monitor this cellular behaviour live under *in vivo* conditions. So far, *in vivo* monitoring of microglial phagocytosis has only been achieved in the neocortex.[58] Live visualization of microglial motility and phagocytosis in deep hypothalamic areas is a technical challenge that must be overcome. One possible solution could be to access the hypothalamus from the ventral surface of the brain using multiphoton microscopy.[59]

Hypothalamic microglial reactivity and obesity

As the centre of metabolic control, the mediobasal hypothalamus rapidly responds to metabolic challenges. This capacity is reflected by the observation that even 1 day after mice or rats are started on a HFD, microglial reactivity increases in the mediobasal hypothalamus, as do levels of proinflammatory cytokines such as tumour necrosis factor (TNF).[18] These inflammatory responses persist under this dietary condition and are caused primarily by the hypercaloric diet. This inflammatory response is not the consequence of weight gain, as obese monogenic leptin receptor mutant *db/db* and melanocortin receptor 4 (MC4R) knockout mice fed a standard chow diet do not show reactive microglia in any brain area.[60] Moreover, microglial activity in monogenic *ob/ob* mice is below the level in chow-fed wild-type mice.[60] Restoring the leptin signal in *ob/ob* mice also restores microglial activity to wild-type levels.[60] This association could be a direct result of expression of the leptin receptor on microglia.[56,61] The immunoreactivity of the microglial scavenger receptor CD68 is also upregulated in the ARC under HFD conditions,[60] which suggests that under these conditions, either more cellular debris is produced by the local microenvironment or that pathogen intrusion from the periphery is increased.

What is the primary driving force of hypothalamic microglial activation in DIO? Candidates include a variety of circulating factors. One candidate is overloaded long-chain saturated fatty acids (SFAs). SFAs are produced under HFD conditions via toll-like receptor 4 and myeloid differentiation factor 88 signalling,[62] which are expressed by activated microglia.[17] Nevertheless, SFAs might also act directly on neurons. Deletion of myeloid differentiation factor 88, which is a downstream signal effector of toll-like receptor 4, in the CNS of obese mice reduced activation of IκB kinase (IKK) in the hypothalamus without changing the levels of proinflammatory cytokines such as TNF and IL-6.[18] This finding indicates that hypothalamic microglial reactivity during early stages of a hypercaloric challenge is a sign of neuronal dysfunction that drives microglia to initiate protective actions.[18] In this case, microglial activation is a consequence of alterations in hypothalamic neurons at the earliest stage of obesity. However, in *in vitro* conditions, hypothalamic neuronal cell cultures do not respond to SFAs, which suggests that hypothalamic neuronal inflammation in DIO is secondary to the action of SFAs on non-neuronal cells.[63]

In addition to SFAs, glucose and/or fructose might be other key nutrient factors that regulate microglial activity via the hexose transporter GLUT5,[64] which is expressed on microglia but is not present on neurons.[65,66] To effectively increase microglial activity both lipids and glucose are required during a hypercaloric challenge. Microglial activity is not higher in chow-fed *db/db* mice with severe hyperglycaemia than in chow-fed wild-type mice; however, microglial activity is increased if *db/db* animals are fed a HFD.[60] In addition, other circulating factors (such as fibrinogen, an important factor in the development of the metabolic syndrome) have stimulatory effects on perivascular macrophages.[40] Ultimately, long-term and sustained activation of microglia might result in a negative feedback loop that increases the risk of impairment of these cells. This situation might be similar to what is described in animal models of Alzheimer disease, where innate immune mechanisms that result in long-term and sustained expression of proinflammatory cytokines such as IL-12 and IL-23 exacerbate the pathology of Alzheimer disease.[22,23,67,68] However, brain samples from patients with Alzheimer disease show microglial senescence.[69] Such impairment of microglia might also include a loss of trophic functions, possibly mimicking conditions of a microglia-specific lack of brain-derived neurotrophic factor, which is important for key physiological brain functions in learning and memory via the promotion of learning-related formation of synapses.[70]

The composition of the gut microbiota differs between lean and obese individuals, in addition, a high-fat, low-fibre, Western-style diet dramatically affects the composition of the human gut microbiota.[71] Whether the gut microbiota has a causative role in obesity[72] or if endotoxins can travel from the intestines to the hypothalamus and stimulate local immune responses is currently unclear.

In addition to blood-borne factors, factors derived from astrocytes and neurons, such as ATP, are potential stimuli for microglia. At the molecular level, ATP has been identified as an inducer of microglia activation through purinoceptors P2X or P2Y.[73,74] For example, studies in leeches have demonstrated that innexin membrane channels that release ATP from glia are required for migration and accumulation of microglia after nerve injury, an effect that could be blocked by arachidonic acid.[75,76] Furthermore, chemokines derived from astrocytes and neurons might also have a role in regulating microglial activity. Within the brain, microglia and non-parenchymal macrophages such as perivascular macrophages express CX3CR1 and receive tonic, inhibitory inputs from fractalkine (CX3CL1) released by neurons.[77] In the obese-prone mouse, hypothalamic expression of fractalkine can be stimulated rapidly, even after 1 day of a HFD.[78] In rats, administration of fractalkine to the paraventricular nucleus, which hosts the preautonomic neurons that are in control of cardiovascular function, can decrease blood pressure and increase the heart rate.[79]

A study published in 2014 that utilized chimeric mice harbouring GFP^+ bone marrow showed that a HFD could stimulate recruitment of peripheral GFP^+ myeloid into the CNS, which might contribute to the increased number of microglia in the hypothalamus.[80] In addition, it was found that some of the CNS-recruited cells along blood vessels were not immunoreactive for AIF-1.[80] However, studies relying on bone marrow chimeric mice produced without shielding the head during lethal irradiation (which is a precondition for successfully generating chimeric animals) must be interpreted with caution. Firstly, head irradiation leads to severe changes in brain homeostasis, including artificially opening the blood–brain barrier and inducing proinflammatory and chemoattractant factors that facilitate recruitment of peripheral haematopoietic cells.[29,81,82] Secondly, as the recruitment of peripheral myeloid cells into the CNS is highly specialized and temporally dynamic, the ratio of peripherally derived versus resident myeloid cells might

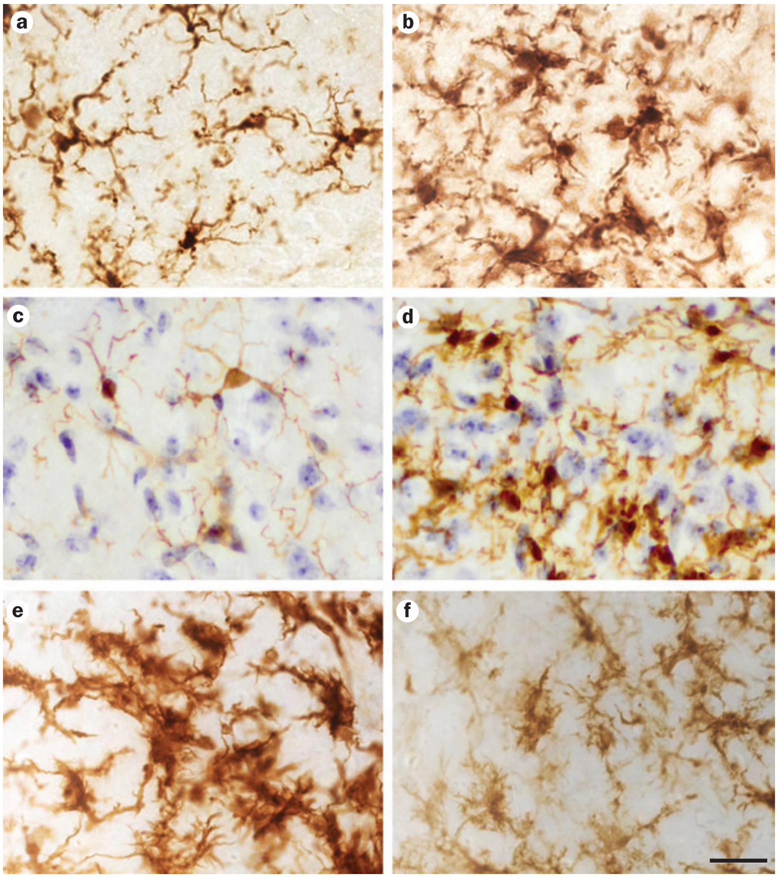

Figure 3 | Morphological comparison of microglial reactivity in diet-induced obesity as visualized by AIF-1-ir. **a** | Reactivity in chow-fed mice. **b** | Reactivity in 8-month old mice fed a high-fat diet. Peripheral nerve injury in **c** | control mice and **d** | injured mice. **e** | Mediobasal hypothalamus injected with lentivirus carrying an empty vector at a concentration of 10^8/ml, and 1 µl was injected in the mediobasal hypothalamus of wild-type C57BL/6 mice. **f** | Mediobasal hypothalamus mechanically injured with a 32 gauge needle used for virus injection. The high-fat diet induced microglial reactivity, visualized by ramification and soma size, is comparable to those by peripheral nerve injury or mechanical injury, but much less than those activated by infectious lentivirus. Scale bar: 20 µm. Abbreviation: AIF-1, allograft inflammatory factor 1.

change over time, for example, upon consumption of a HFD. A site-specific histological analysis of the hypothalamus is required for a proper analysis of myeloid cells *in situ,* including the assessment of their relationship to neuroanatomical structures. Additional studies are needed to provide the direct evidence required to define the number and function of the recruited peripheral myeloid cells in the hypothalamus. These studies should be able to determine how these cells function after infiltration, if they acquire a full microglial phenotype, and, if so, at what stage and time point, and whether peripherally derived myeloid cells confer beneficial or detrimental effects.

In the periphery, macrophages can acquire different inflammation-related phenotypes termed M1 ('classic', induced by IFN-γ and lipopolysaccharides) and M2 ('alternative', induced by IL-4 and IL-13), mediating host defence and supporting anti-inflammatory processes, respectively.[83,84] Some evidence suggests that microglia in the CNS can acquire similar phenotypes *in vitro* and *in vivo*.[85,86] However, resident microglia are functionally distinct from blood-derived phagocytes,[87] leading to the conclusion that the M1 and M2 phenotypes might represent only the extreme ends of a continuum of activation.[88] Of note, research into macrophage biology over the past 20 years has led to the M1/M2 concept for macrophages being abandoned by some researchers.[89]

Irrespective of how microglial phenotypes might ultimately be classified (we propose not being dogmatic and to follow the recommendations to relinquish the M1/M2 framework for microglia as has been proposed for macrophages), these cells produce a large and diverse selection of mediators. In the hypothalamus of mice with long-term (that is, at least 8 months) DIO, microglia are thought to be maximally activated. We found that the DIO-induced morphological changes in the hypothalamic microglia and macrophages were similar to those of reactive microglia induced by peripheral nerve injury in the dorsal horn of the spinal cord (lumbar 4–6), and were less extensive than changes induced by virus-activated microglia and/or infiltrating macrophages in the mediobasal hypothalamus (Figure 3). This observation indicates that the DIO-stimulated microglia–macrophage reactivity does not reach the maximal activation capacity of both populations, defining a low-grade reactivity that is beyond the classification scope of M1 and M2 nomenclature. To determine the exact roles of the low-grade activated microglia and macrophages in the hypothalamus in a hypercaloric environment, a systemic phenotyping of the reactive microglia and macrophage population in the hypothalamus over time is required to compare the gene and protein expression profiles. Data on hypothalamic myeloid cell populations, however, are still largely missing. Moreover, one must be aware that microglia isolated by cell-sorting methods are exquisitely sensitive to disturbances, even under residential *in vitro* conditions, which would possibly influence data interpretation.

Until 2 years ago, genetic manipulation of microglia *in vivo* was difficult because no appropriate specific transgenic mouse was available owing to most of the existing Cre mouse lines encompassing microglia, macrophages, dendritic cells and natural killer cells.[90] However, studies from different research groups published in the past 2 years have been successful in generating microglia-specific genetic manipulations by means of tamoxifen-inducible Cre recombination.[70,91] With this approach, the majority of the microglia in the brain maintain the 'manipulated' state, while peripheral monocytes return to wild-type phenotypes due to their naturally high turnover rates. As has been discussed in this Review, however, it is questionable whether microglia in the leaking blood–brain barrier regions, such as those in the median eminence and ARC, can maintain their 'manipulated' phenotype. Another interesting genetic model is the CD11b–HSVTK transgenic mouse. These mice express the herpes simplex virus thymidine kinase in macrophages and microglia, which enables transient depletion of resident microglia in the CNS, including the hypothalamus, for a maximum of up to 3–4 weeks by local administration of ganciclovir (an antiviral medication).[92,93] The transient depletion of microglia is followed by repopulation of peripherally derived myeloid cells replacing resident microglia upon ablation.[30]

Hypothalamic astrocyte activation

Hypothalamic astrocytes and metabolic sensing

Astrocytes process large parts of the energy supplied to neurons by converting glucose to lactate and lipids to ketone bodies via monocarboxylate transporters.[94–96] In the adult brain, astrocytes can use fatty acids instead of glucose as their main energy substrate under two specific conditions—fasting and/or when fed a HFD.[94] In addition to their role as nutrient sensors and converters, astrocytes also express receptors for metabolic hormones, such as leptin, ghrelin and insulin.[97–99] Astrocytic leptin signalling contributes to the regulation of food intake by leptin from the CNS, as proven by a study showing that after deletion of astrocyte-specific leptin receptors, leptin inhibition of food intake was diminished, and the effects of ghrelin, a hormone that stimulates hunger and is the natural antagonist of leptin, were elevated.[100] With their expression of type II iodothyronine deiodinase,[101] astrocytes, together with tanycytes in the hypothalamus, are also important for transporting and converting thyroid hormone T_4 into T_3 for metabolic regulation.[102,103]

The heterogeneity of astrocytes in the CNS might be more complicated than that of microglia, as glial fibrillary acidic protein (GFAP) has at least nine splice variants described in distinct types of astrocytes in human and rodents studies.[104] However, the heterogeneity could also be due to the lack of a proper understanding of astrocyte biology. The astrocyte population in the hypothalamus is also thought to be heterogenic, as reflected by the nuclei-specific increase of astrocyte reactivity under HFD conditions.

Hypothalamic astrocytic reactivity and obesity

Upon exposure to a HFD, the hypothalamus undergoes astrocytosis as rapidly as it undergoes microgliosis.[18] This astrocytosis prevents neurons from communicating with circulating metabolic feedback factors, such as leptin,[105] which might partially contribute to the central mechanisms of disturbed leptin action, referred to as 'leptin resistance'.[106] Proinflammatory factors produced by astrocytes, such as IL-6, also contribute to the hypothalamic inflammatory profile. Interestingly, although transgenic mice with overexpression of IL-6 in astrocytes develop astrocytosis, they are resistant to DIO, without alterations in their food intake.[107] Apparently, astrocytosis induced by IL-6 shifts CNS control centres toward increased systemic energy metabolism during HFD exposure, which suggests that astrocytic changes are beneficial to the hypothalamic control of systemic metabolism.[107] Nevertheless, in a case study, a patient with an astrocytoma limited to the hypothalamus was hyperphagic and had obesity, which provides an example of a translational role for astrocytes in metabolism.[108]

As in the context of microglia, the question remains: how does a HFD stimulate astrocytosis in the CNS?

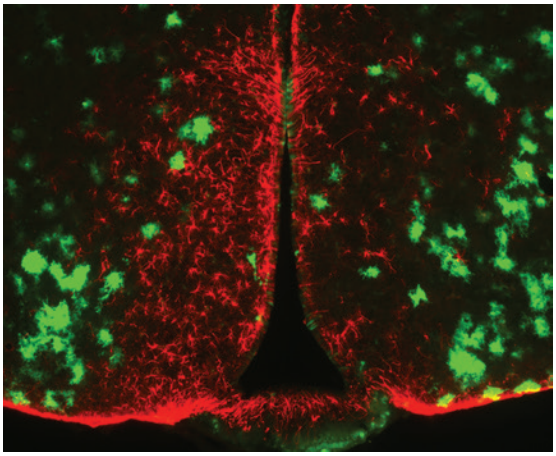

Figure 4 | The distribution pattern of the GFP⁺ astrocytes (green) and GFAP-ir astrocytes (red) in hGFAP-GFP transgenic mouse hypothalamus. Abbreviations: GFAP-ir, immunofluorescent staining of glial fibrillary acidic protein; GFP, green fluorescent protein.

One explanation is a shift from lactate to ketone bodies that might occur with a hypercaloric diet. The exact HFD-related stimulus that causes astrocyte activation necessitates a systemic phenotyping approach similar to that described for microglia to determine the differences in astrocytes between lean and obese conditions. For this approach, existing Cre mouse lines with mouse *Gfap* or human *GFAP* promoters could be used. Interestingly, when GFP is expressed under the influence of the human *GFAP* promoter, only the protoplasmic astrocytes are positive for GFP, whereas the HFD-stimulated astrocytosis mainly takes place in fibrous astrocytosis (Figure 4).[109] Cre lines driven by promoters of other pan-astrocyte markers, such as the aldehyde dehydrogenase 1 family member L1 and glutamate transporters GLT1 and GLAST that are expressed in diverse astrocyte populations, must also be considered in the study of hypothalamic astrocyte functions. However, sophisticated tools to study the diverse functions of heterogeneous astrocytes in the hypothalamus are currently lacking.

Hypothalamic vasculature and obesity

As discussed in a previous section, the hypothalamus has a unique, fenestrated capillary system in the median eminence. The overall physiological function of this special vasculature is still not fully understood. This vasculature might be helpful for some glial functions, such as the uptake of nutrients by astrocytes and the removal of debris by microglia. Such highly permeable vasculature could also help the ARC neurons to have improved access to circulating metabolic signals. A study using a real time *in vivo* imaging approach has shown that fluorescently labelled ghrelin can diffuse through the fenestrated capillaries of the median eminence and rapidly bind to the nearby POMC and NPY neurons.[59] One can imagine that if this neuron–blood communication is impaired under HFD conditions, the normal satiety feedback signals would not reach the metabolic control circuits in the hypothalamus, and the stimulus would not turn off, leading to excessive hunger or increased appetite.

Mice with DIO that are fed a long-term HFD have a considerably increased blood vessel density and length, which is an indication of hypothalamic angiopathy.[19] Consistent with this finding, patients with type 2 diabetes mellitus have increased numbers of pre-capillary arterioles in the infundibular nuclei (equivalent to the ARC in rodents),[19] which is reminiscent of diabetic retinopathy and suggests that a functional involvement of the hypothalamic vasculature could be involved in the late stages of the pathogenesis of the metabolic syndrome.

The vascular endothelial growth factor (VEGF) is a key molecular angiogenic factor for neovascularization and hypervascularization. In some brain areas, such as the hippocampus, astrocytes are the major producers of VEGF, whereas in the hypothalamus, tanycytes are a source of VEGF.[110] In mice (particularly in conditions of food deprivation), tanycytes express VEGF-A, which targets microvessel loops in the median eminence and promotes microvessel permeability and tight junction complex reorganization in the median eminence and ARC, which results in improved access to metabolic substrates in this brain region.[110] Whether astrocytosis is the source of VEGF that promotes angiogenesis under HFD conditions as well as the exact purpose of angiogenesis in this region remain to be determined.

Neurons and proinflammatory signals

The roles of the TNF and IKK-β–NF-κB pathways

In response to a HFD challenge, reactive microglia respond to both acute and chronic hypothalamic inflammatory changes by producing proinflammatory cytokines and by inducing inflammatory responses in neighbouring neurons.[17,18,111] Of the proinflammatory cytokines, TNF has gained most of the attention. The link between TNF and obesity was established two decades ago in studies of white adipose tissue, in which blocking TNF signalling improved insulin sensitivity in obese rats.[112] A lack of TNF is, therefore, expected to prevent DIO and/or insulin resistance. TNF-deficient mice show no resistance to hyperphagic obesity induced by aurothioglucose, but they do have reduced fasting plasma levels of glucose and insulin, which indicates that TNF might have a role in lipid and glucose metabolism in mice with DIO.[113] Interestingly, mice with a double knock out for the two TNF receptors, TNFR1 and TNFR2, are not prevented from developing obesity-induced insulin resistance.[114] These knockout mice are even more susceptible to DIO than wild-type mice, with reduced energy expenditure, which highlights the complexity of downstream TNF signals in mediating TNF effects.[114] More than 20 members of the TNF receptor superfamily have been identified, but their involvement in proliferation, survival, differentiation and apoptosis is still under investigation.[115] The complexity of TNF signalling in neurons is related to the crosstalk between receptors for different ligands of the receptor superfamily members and the crosstalk between TNF receptors and other cell receptors, such as insulin-like growth factor 1, that have a role in neurodegenerative diseases.

The most intensively studied TNF downstream signalling pathway in obesity is IKK-β, which is the inhibitor of the nuclear factor κB (NF-κB) signalling pathway[6,116,117] and the receptor activator of NF-κB ligand (RANKL). In the hypothalamus of mice with DIO, signals along the NF-κB pathway, such as Ikbkb and Ikbke, are raised.[18] However, the roles of the IKK-β and NF-κB signalling pathways in obesity are diverse. On one hand, inhibition of IKK-β can reverse obesity caused by hypothalamic autophagy,[118] and deactivation of NF-κB can counteract obesity-related hypertension.[119] Hypothalamic neural stem cells can also be depleted under HFD conditions via the activation of IKK-β–NF-κB signalling pathways.[21] Conversely, in other disease models and brain areas, studies suggest TNF and IKK-β–NF-κB signalling have beneficial effects in mediating cell survival and neuroprotective effects.[120] For example, compounds that damage DNA can induce apoptosis via inhibition of NF-κB activity and the rescue of NF-κB activity can prevent brain damage induced by ischaemia.[121]

In addition, functional NF-κB complexes are present not only in neurons but also in astrocytes, microglia and oligodendrocytes, which could explain the diverse functions of TNF and IKK-β–NF-κB, given that these cell types express a variety of TNF receptors. *In vitro* studies have shown that the strongest NF-κB activity in response to TNF is in non-neural cells such as microglia, which are activated by TNF autocrine functions.[122,123] Thus, it might be that under certain circumstances, increased NF-κB activity also occurs in microglia, in addition to driving inflammatory responses in neurons. Therefore, cell specificity needs to be considered when interpreting NF-κB data from the hypothalamus given the heterogeneity of hypothalamic cell types. TNF-induced neuronal production of chemokines (such as CXCL1 and CXCL10) is mediated by IKK-β and NF-κB.[123] That these chemokines can act on microglia implies that a feedback loop might exist between neurons and microglia regarding their immune communication.

Of note, many of the studies on NF-κB activity were performed in experimental pathological conditions. NF-κB activity can also be raised under physiological conditions, for example, in muscle tissue following acute exercise in lean individuals and individuals with obesity but not diabetes mellitus.[124] More physiological studies are required to completely understand NF-κB pathways in the hypothalamus.

Neuronal compartments

Hypothalamic pathways need to maintain plasticity to remodel the neural circuitry in response to metabolic challenges.[125,126] Diverse mechanisms are involved in altering synaptic communication and influencing mitochondrial production of ATP is one essential step.[127–129] Mitochondrial physiology in neurons is different from that of other cell types because of the long distances that neural processes extend from the cell body. Given these distances, mitochondria must travel along axons toward the synapse.[127–129] The maintenance and normal functioning of neuronal synaptic communication relies on

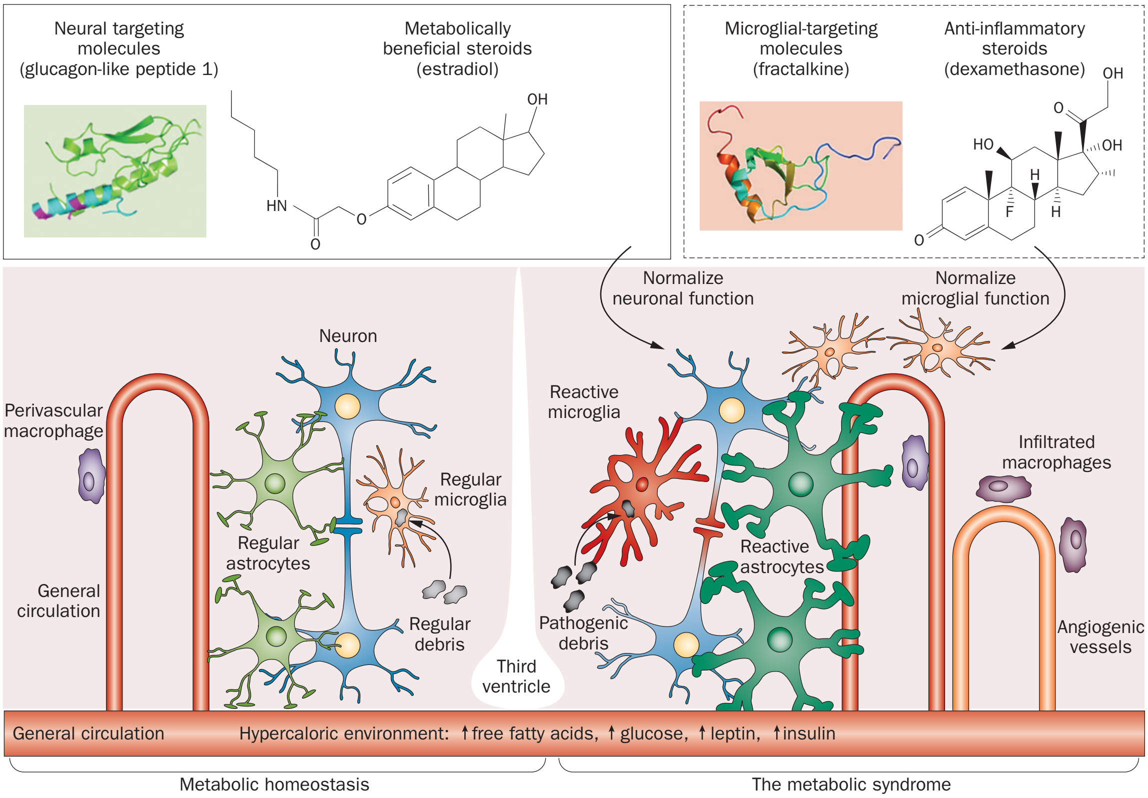

Figure 5 | The diverse cell populations in the local microenvironment of the mediobasal hypothalamus and their respective transitions from basal conditions to reactive stages in the metabolic syndrome. With exposure to a hypercaloric environment, microglia and astrocytes rapidly enter reactive states. Peripheral monocytes might also infiltrate this area as part of the local microenvironment immune response. In the long term, the communication between neurons and the general circulation is hampered by astrocytosis and angiogenic factors derived from the local microenvironment, which drive angiogenesis. The cell-specific antiobesity therapeutic strategy of targeting cells expressing the glucagon-like peptide 1 receptor with metabolically beneficial estradiol is effective, depending on the specific intercellular and intracellular mechanisms. This strategy confers the benefits of estrogen without the unwanted adverse effects. To specifically target reactive microglia, a conjugate in which fractalkine can carry anti-inflammatory steroids (such as dexamethasone) into the microglia is proposed.

mitochondrial bioenergetics (ATP production) as well as mitochondrial motility. Regular functions of mitochondria, such as Ca^{2+} loading, are tightly coupled to the endoplasmic reticulum,[130] and endoplasmic reticulum stress directly leads to malfunctioning mitochondria.[131] In mice with DIO, the density of mitochondria and their interactions with endoplasmic reticulum in POMC neurons are decreased.[18,132,133] In AgRP neurons, deletion of mitofusin 2, a key molecule that controls mitochondrial physiology, can decrease electrical activity of these neurons following exposure to a hypercaloric diet.[134] Deletion of mitofusin 2 in POMC neurons leads to primary endoplasmic reticulum stress, causing defective α-melanocyte-stimulating hormone processing, leptin resistance and obesity.[132] These data emphasize that mitochondria–endoplasmic reticulum interactions in neurons have a crucial role in the maintenance of normal energy homeostasis and adequate responses to metabolic challenges.

The reasons for malfunctioning mitochondria–endoplasmic reticulum interactions are not completely understood. On the basis of the evidence that cytokines trigger endoplasmic reticulum stress via the NF-κB pathway,[135] one could propose a causal link between glial-derived cytokines and mitochondrial–endoplasmic reticulum dysfunction. This hypothesis is supported by the fact that in mice with long-term DIO, the quantity of POMC neurons in the hypothalamus is reduced by 25% in comparison with lean mice.[18] Mitochondrial–endoplasmic reticulum dysfunction can cause neurodegenerative changes in the hypothalamus, as mitochondrial dysfunction has been implicated in neurodegenerative diseases, such as Alzheimer disease and Parkinson disease, and normalizing mitochondrial function has been considered as a therapeutic target.[136]

Translational aspects

The ultimate goal of studying animal models of metabolic disorders is to understand the pathophysiology of human diseases and search for effective therapeutic approaches. With respect to translational studies on hypothalamic reactive responses to metabolic disorders, one of the main approaches is to collect post-mortem brain material. This approach is justified by the fact that there are already indications for the loss of anorexigenic POMC neurons in the hypothalami of mice with long-term DIO[18] and patients with type 2 diabetes mellitus.[137] However, the unbiased interpretation of results obtained from this tissue is still challenging, as information on the patient is not always complete, and the material is usually obtained from diseased and/or elderly people. Obviously, it is necessary to consider post-mortem delays in tissue collection, the patient's cause of death and their entire health history, including the history and severity of any disease (but especially obesity and diabetes mellitus), sex, age and comorbid conditions. Another option is to use some of the post-mortem human brain tissue from patients to generate primary cultured human microglia. However, some concerns have been raised about subsequent data interpretation.[138,139] Due to the aforementioned difficulties, more clinical evidence is needed to improve the study of hypothalamic inflammation in human obesity, which involves joint efforts of clinicians, basic researchers and neuropathologists.

Implications for treating obesity

Overall, the temporal and spatial changes of each single-cell population and cellular signalling pathway that determine the magnitude of the hypothalamic pro-inflammatory responses to hypercaloric challenges are not fully understood. To investigate the complexity of microglia–neuronal interactions, we need to connect each glial inflammatory response to its final effect on neuronal function. With the evidence presented here that the hypothalamic neural–glial network is immunologically activated under DIO conditions, one can propose that pharmacotherapies should be designed to intervene in and/or modulate the immune response within the dysregulated neuro–glial network by specific targeting of microglial populations. The feasibility of this concept has already been proven in studies in which estrogen was conjugated to GLP-1.[140] Estrogen and GLP-1 are known to have beneficial effects on metabolic disorders; however, high dosages of estrogen also have adverse effects, such as exacerbating oncogenic potential, for example, when acting on the uterus.[141]

When conjugated to GLP-1, estrogen can act only on cells that express both estrogen receptors and GLP-1 receptors, such as hypothalamic neurons that control food intake and energy balance. The estrogen-sensitive cells in the uterus are not targeted, as they do not express the GLP-1 receptor. This conjugate technique provides promising treatment options as it has clear beneficial effects in mice with DIO.[140] On the basis of this concept of a cell-specific pharmacotherapy, microglia could be specifically targeted with dexamethasone to normalize the microglial production of cytokines and the phagocytic capacity. In macrophages, dexamethasone promotes the phagocytic capacity and reduces the lipopolysaccharide-induced production of cytokines.[142] Preventing dexamethasone from affecting the hypothalamic neurons and other peripheral organs, such as muscle and liver, would be a prerequisite for this treatment approach, as studies have shown that dexamethasone can induce insulin resistance systemically[143,144] or by acting on hypothalamic NPY neurons.[145] Fractalkine would be one possible option for a microglia-targeting molecule that could carry dexamethasone specifically into the microglia. The receptor for fractalkine is uniquely expressed by microglia in the CNS, and it has a neuroprotective function (Figure 5). Other neuron-specific and microglia-specific targeting molecules that can carry compounds into different cell populations to combat inflammation are expected to be identified by additional studies investigating the relationship between hypothalamic neural–glial dysfunction and obesity.

Conclusions

The hypothalamus is the key brain region responsible for the regulation of energy balance and glucose metabolism. Accumulating evidence indicates that hypothalamic neuropathy is the leading candidate that could contribute to the pathogenesis of obesity and diabetes mellitus. The neuronal dysfunction, however, is associated with the innate immune reactivity generated by the mutual interaction between neurons and other cell populations in the hypothalamus, while handling metabolic challenges from the hypercaloric environment. Thus to treat obesity and associated metabolic disorders, multi-targeting pharmacotherapy is needed for normalizing each affected cell population individually in the disordered hypothalamic microenvironment.

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