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Review article

# Emerging roles for hypothalamic microglia as regulators of physiological homeostasis

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ABSTRACT

The hypothalamus is a crucial brain region that responds to external stressors and functions to maintain physiological homeostatic processes, such as core body temperature and energy balance. The hypothalamus regulates homeostasis by producing hormones that thereby influence the production of other hormones that then control the internal milieu of the body. Microglia are resident macrophages and phagocytic immune cells of the central nervous system (CNS), classically known for surveying the brain's environment, responding to neural insults, and disposing of cellular debris. Recent evidence has shown that microglia are also responsive to external stressors and can influence both the development and function of the hypothalamus in a sex-dependent manner. This emerging microglia-hypothalamic interaction raises the intriguing notion that microglia might play an unappreciated role in hypothalamic control of physiological homeostasis. In this review, we briefly outline how the hypothalamus regulates physiological homeostasis and then describe how this literature overlaps with our understanding of microglia's role in the CNS. We also outline the current literature demonstrating how microglia loss or activation affects the hypothalamus, and ultimately homeostasis. We conclude by proposing how microglia could be key regulators of homeostatic processes by sensing cues external to the CNS and transmitting them through the hypothalamus.

## 1. Introduction

The hypothalamus is a powerful brain region that is responsive to external stressors and is critical for maintaining homeostatic processes, including thirst, energy balance, mood, circadian rhythm, reproduction, parturition, and lactation. The hypothalamus regulates physiological homeostasis by releasing trophic hormones that serve to connect the nervous system to the peripheral endocrine system found throughout the body. Microglia, the resident macrophages and phagocytic immune cells of the central nervous system (CNS), survey their environment, respond to neural insults, and dispose of cellular debris. In addition, microglia can sense external stressors, including insults external to the CNS (e.g., infection, glucocorticoids, gut-brain inputs, etc.) (reviewed in ref. Frank et al., 2015; Frank et al., 2019; Sorrells and Sapolsky, 2007; Rosin and Kurrasch, 2018; Thion et al., 2018) and those external to the body (e.g., ozone, diesel exhaust, air pollution, environmental contaminants, etc.) (Bolton et al., 2013; Bolton et al., 2017; Levesque et al., 2013; Levesque et al., 2011, reviewed in ref. Rosin and Kurrasch, 2018), causing them to interact with neighboring neurons to control their local environment. Given that recent data show microglia play important roles both in hypothalamic development and function throughout life, in this review we propose that perhaps these two systems intersect, whereby microglia are sensitive to some external inputs and can transmit these cues to neighboring hypothalamic neurons to also control physiological homeostasis.

## 2. Hypothalamic control of body homeostasis

The hypothalamus is a dynamic and key brain region that mediates key physiologies such as our fight or flight response. It is also critical for maintaining homeostatic processes, such as fluid-balance, body temperature, energy regulation, and metabolism (reviewed in ref. Nesan and Kurrasch, 2016; Berthoud, 2002; Hill et al., 2008; Sousa-Ferreira et al., 2014; van Dijk et al., 2011). The hypothalamus can also influence specific behaviours, including mood, physical activity, diurnal activity, reproduction, parturition, and lactation, amoung others (Li et al., 1998; reviewed in ref. Nesan and Kurrasch, 2016; Fontes et al., 2011; Bandler et al., 2000). The hypothalamus acts to provide a link between the

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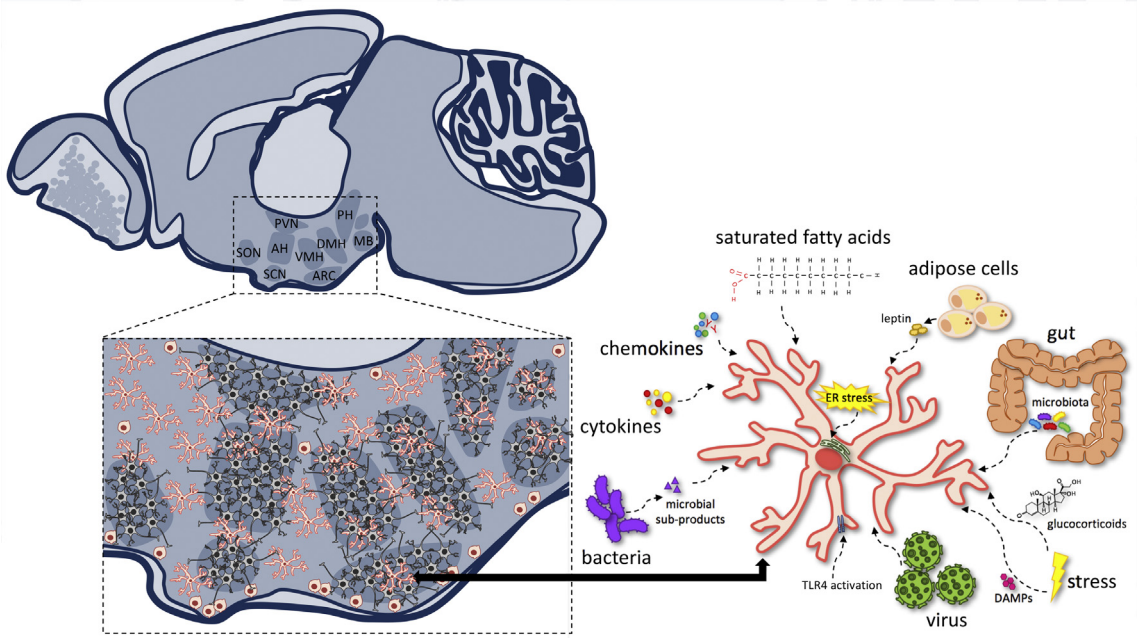


**Fig. 1.** Microglia act as sensors to regulate hypothalamic development and function. Schematic diagram of the mature hypothalamus and the different pathogens, secreted factors, and signaling molecules that act on microglia in the hypothalamus to regulate homeostasis both locally in the brain and peripherally in the body. Top left, the nuclei in the mature hypothalamus as depicted in a sagittal section through the brain. Specifically, from left to right, the supraoptic nucleus (SON), anterior hypothalamic nucleus (AH), suprachiasmatic nucleus (SCN), paraventricular nucleus (PVN), ventromedial hypothalamic nucleus (VMH), arcuate nucleus (ARC), dorsomedial hypothalamic nucleus (DMH), posterior hypothalamic nucleus (PH), and the mammillary body (MB). Bottom left, the hypothalamus is depicted at high magnification, where neurons (grey cells) are clustered in the specific hypothalamic nuclei and microglia (peach cells) are spread evenly throughout the hypothalamus. Right panel, microglia are detecting and reacting to signals from viruses, toll-like receptor 4 (TLR4) activation, microbial sub-products from bacteria, cytokines, chemokines, saturated fatty acids, endoplasmic reticulum (ER) stress, leptin secreted from adipose cells, microbiota from the gut, and stress in the form of glucocorticoids and danger associated molecular patterns (DAMPs). Sagittal section through the mature hypothalamus is adapted from Nesan and Kurrasch (2016).

nervous system and the endocrine system via stimulation of the pituitary gland, typically referred to as the hypothalamic-pituitary axis. Structurally, the hypothalamus is divided into three rostrocaudal regions: the anterior, tuberal, and mammillary hypothalamus. The anterior hypothalamic nuclei include the supraoptic nucleus (SON), suprachiasmatic nucleus (SCN), anterior hypothalamic nucleus (AH), and the anterior portion of the paraventricular nucleus (PVN). The tuberal hypothalamus is comprised of the posterior portion of the PVN, ventromedial hypothalamic nucleus (VMH), dorsomedial hypothalamic nucleus (DMH), arcuate nucleus (ARC), and the lateral hypothalamus (LH). Finally, the mammillary hypothalamus contains the posterior hypothalamic nucleus (PH), and the mammillary body (MB) (Fig. 1) (reviewed in ref. Nesan and Kurrasch, 2016; Berthoud, 2002).

The tuberal hypothalamus is involved in the regulation of a number of complex homeostatic processes, including thirst, food intake, reproduction, parturition, and lactation (Li et al., 1998, reviewed in ref. Nesan and Kurrasch, 2016; Berthoud, 2002; Hill et al., 2008; Sousa-Ferreira et al., 2014; van Dijk et al., 2011). Outside of these physiological homeostatic processes, the tuberal hypothalamus is also involved in emotional outputs, such as aggression and defensiveness, making it a complex and dynamic region of the hypothalamus (reviewed in ref. Nesan and Kurrasch, 2016; Fontes et al., 2011; Bandler et al., 2000). Consistent with its role in controlling a wide variety of regulatory processes to maintain homeostasis, the hypothalamus consists not only of intra-hypothalamic projections, connecting one nucleus to another, but also projections linking hypothalamic nuclei to distant brain regions. For example, the PVN receives inputs from the cortex and brainstem, and sends projections to the anterior pituitary to regulate the release of trophic hormones (Biag et al., 2012; Bouyer and Simerly, 2013; Sawchenko and Swanson, 1982). Wakefulness and sleep, in contrast, are thought to be controlled by the LH and its connections to the cortex and basal forebrain (Hahn and Swanson, 2010). Although the VMH is known to play a role in satiety signaling, it also participates in aggression and sexual behaviours, which is mediated via circuitry to the DMH, PVN, and the anterior hypothalamus (Canteras et al., 1994; ter Horst and Luiten, 1986, reviewed in ref. Saper and Lowell, 2014). Outside of the hypothalamus, the VMH sends projections to the amygdala and the midbrain (Canteras et al., 1994; Choi et al., 2005). Meanwhile, in the mediobasal tuberal hypothalamus, the ARC contains two widely studied neuronal populations that regulate energy and glucose homeostasis, namely the agouti-related peptide/neuropeptide Y (AgRP/NPY) and pro-opiomelanocortin (POMC) neurons. To govern energy and/or glucose balance, these populations project to the PVN, DMH, and LH within the hypothalamus, in addition to brain regions outside of the hypothalamus, including the dorsal raphe nucleus within the brainstem (Bouyer and Simerly, 2013; Bouret et al., 2004; Sim and Joseph, 1991). Together, numerous studies have shown that hypothalamic nuclei and their projections act in concert to connect the CNS to the endocrine system and play a critical role in the regulation of body homeostasis.

### 2.1. Feeding behaviour and energy balance

As an example of how microglia activation might intersect with hypothalamic homeostatic mechanisms, first we use energy balance as a case in point. Below, we outline what is known about hypothalamic control of feeding behaviours, followed by a discussion of the emerging literature demonstrating a role for microglia in this process. The involvement of the hypothalamus in the control of energy balance is of particular interest given the rise in the prevalence of obesity and neuroendocrine disorders involving improper metabolism. Since this topic is heavily reviewed elsewhere (reviewed in ref. Berthoud, 2002; Le Thuc et al., 2017; Avalos et al., 2018; Garcia-Caceres et al., 2019; Cakir and Nillni, 2019), here we provide just a brief overview.

Discrete subsets of hypothalamic neurons (e.g., AgRP/NPY and POMC) are responsible for sensing and communicating energy availability by detecting secreted molecules in the blood circulation. The hypothalamus then uses this information to either induce eating or satiety to maintain energy balance. The first descriptions identifying the hypothalamus as a regulator of metabolic homeostasis came from early studies showing that physical or chemical damage to specific nuclei within the hypothalamus, including the PVN, VMH, and DMH, cause hyperphagia (i.e., excessive hunger) and obesity (Brobeck, 1946; Hetherington, 1944; Hetherington, 1942). In contrast, lesions to the LH lead to a reduction in food intake, or hypophagia (Anand and Brobeck, 1951). Together, studies such as these provide the foundation for our understanding that the regulation of food intake and body weight is controlled by specific regions within the hypothalamus. Decades later, these early discoveries were further supported by the findings that circulating metabolic hormones, including leptin, ghrelin, and insulin, signal to and communicate with these same nuclei in the hypothalamus (Woods et al., 1979; Zhang et al., 1994; Kojima et al., 1999). For example, ghrelin is produced by the stomach to stimulate appetite and increase food intake (reviewed in ref. Cohen et al., 1999), while leptin and insulin, produced by adipose cells or the pancreas, respectively, signal to decrease food intake (reviewed in ref. Nunziata et al., 2019; Davis et al., 2010). Technological advancements, including a wide-range of mouse genetic models, optogenetic stimulations, ablation studies, and cell-sequencing techniques, are continually contributing to our understanding of the specific cell types within the hypothalamus that facilitate signaling through feeding circuity. This is particularly true within the ARC given its involvement in transforming metabolic cues into neuronal signals.

The ARC is located in the ventral floor of the tuberal hypothalamus, where it borders the median eminence (ME) – a circumventricular organ (CVO) that is comprised of fenestrated capillaries that facilitate the diffusion of specific molecules found in the blood. This location underlies the ARC's ability to send dendrites into the ME to directly access nutrients and secreted signals that circulate in the blood and inform on the peripheral metabolic state (Djogo et al., 2016, reviewed in ref. Garcia-Caceres et al., 2019). Two of the best studied neuronal populations located within the ARC that are crucial for sensing nutritional status, yet act in opposition to regulate appetite and satiety, are the anorexigenic POMC neurons (Gantz et al., 1993) and the orexigenic AgRP/NPY neurons (Tatemoto et al., 1982; Miltenberger et al., 1997; Andrews et al., 2008; Mandelblat-Cerf et al., 2015, reviewed in ref. Avalos et al., 2018). POMC neurons promote energy expenditure and satiety by releasing the neuropeptide alpha-melanocyte-stimulating hormone (α-MSH), which binds to melanocortin receptors (reviewed in ref. Avalos et al., 2018; Mountjoy, 2010). Specifically, following the ingestion of food, a rise in circulating leptin provokes POMC neuronal activation through the formation of reactive oxygen species in POMC neurons (Diano et al., 2011). Once POMC neurons become activated, peptides such as α-MSH are secreted, which functions as a satiety cue and leads to increased energy expenditure through the activation of melanocortin receptors (e.g., MC3R/MC4R) in the PVN and nucleus of the solitary tract (Pinto et al., 2004; Koch et al., 2015, reviewed in ref. Garcia-Caceres et al., 2019; Varela and Horvath, 2012). Interestingly, recent studies using single-cell RNA sequencing (scRNAseq) have uncovered an unappreciated spatial heterogeneity of POMC neurons, with lateral POMC neurons more sensitive to leptin and medial POMC neurons more responsive to glucose (Lam et al., 2017).

In direct opposition, AgRP/NPY neurons reduce energy expenditure and promote food intake, ultimately resulting in weight gain (reviewed in ref. Avalos et al., 2018; Sainsbury and Zhang, 2010). Ablating AgRP neurons using AgRP-targeted diphtheria toxin results in starvation and death in adult mice (Luquet et al., 2005), demonstrating the key role of these neurons in orexigenic behaviors. In contrast, activation of AgRP neurons using designer receptors exclusively activated by designer drugs (DREADD) technology promotes feeding and energy expenditure, and increases fat stores in mice (Krashes et al., 2011). Moreover, optogenetic activation of AgRP neurons in mice show that AgRP neurons can impair insulin-stimulated glucose uptake in brown adipose tissue, ultimately resulting in insulin resistance (Steculorum et al., 2016). This demonstrates the importance of AgRP neurons for both insulin sensitivity and glucose metabolism (Steculorum et al., 2016). Taken together, the opposing activity of POMC and AgRP/NPY neurons are required for proper hypothalamic control of feeding and energy balance, which ultimately affects body homeostasis and can result in neuroendocrine diseases, such as obesity, when proper balance is not achieved.

Indeed, the rates of obesity have nearly doubled since 1980, with current worldwide overweight and obesity levels reaching almost a third of our population (Ng et al., 2014; Stevens et al., 2012, reviewed in ref. Hruby and Hu, 2015). Moreover, childhood obesity is growing at an alarming rate, with one in five school-age children categorized as obese (Ervin et al., 2014). For reasons that remain poorly understood, children of mothers that were obese during pregnancy have higher rates of hyperphagia, increased body mass, higher fat composition, and insulin resistance (Nivoit et al., 2009; Samuelsson et al., 2008, reviewed in ref. Ornoy, 2011; Tamashiro and Moran, 2010), suggesting that maternal nutrient status somehow influences development of hypothalamic circuits. Understanding the mechanisms underlying altered energy balance, whether it be from increased caloric intake, genetic factors, developmental influences, hormonal changes, or a side effect of drug treatment, is key for controlling this growing public health issue. At present, most studies have focused on neuronal populations and altered connectivity. However, given that hypothalamic neuroinflammation is also associated with obesity, there may be an unappreciated role for microglia in the etiology of this disease.

## 3. Microglia contribute to hypothalamic development and function

Early in embryogenesis, erythro-myeloid progenitors (EMPs) in the yolk sac give rise to macrophages that travel into the neuroectoderm and invade the developing CNS to generate microglia (Ginhoux et al., 2010; Gomez Perdiguero et al., 2015; Kierdorf et al., 2013). EMPs begin to express cluster of differentiation 45 (CD45) and C-X3-C motif chemokine receptor 1 (CX3CR1) as they transition through multiple immature cellular stages to generate the macrophages that will go on to become microglia (Kierdorf et al., 2013). The progression from macrophage to microglia also involves a number of cellular changes, including the downregulation of the tyrosine phosphatase receptor CD45 and the onset of expression of microglia-specific marks, such as purinergic receptor p2y, G-protein coupled, 12 (P2RY12) (Butovsky et al., 2014). These changes are thought to occur in response to the signals macrophages receive following entry into the CNS microenvironment. Accordingly, microglia become the tissue-resident phagocytic immune cells of the CNS and are responsible for surveying their surroundings in order to promptly respond to neural insults and dispose of cellular debris. Beyond these classic functions in the brain, embryonic microglia also influence neural development by controlling the balance between neural progenitor maintenance (Antony et al., 2011) and neural progenitor engulfment that signals to end neurogenesis (Cunningham et al., 2013). Moreover, during embryogenesis microglia are involved in cortical neuron differentiation, migration, wiring, and lamination (Aarum et al., 2003; Squarzoni et al., 2014). In the postnatal brain, microglia can mediate both dendritic spine formation (Miyamoto et al., 2016) and synaptic pruning (Paolicelli et al., 2011; Schafer et al., 2012; Tremblay et al., 2010). And perhaps most relevant to the discussion herein, microglia seem to respond to a variety or stressors, including stressors external to the CNS (e.g., infection, glucocorticoids, gut-brain inputs, etc.) (reviewed in ref. Frank et al., 2015; Frank et al., 2019; Sorrells and Sapolsky, 2007; Rosin and Kurrasch, 2018; Thion et al., 2018), as well as those external to the body (e.g., ozone, diesel exhaust,

air pollution, environmental contaminants, etc.) (Bolton et al., 2013; Bolton et al., 2017; Levesque et al., 2013; Levesque et al., 2011, reviewed in ref. Rosin and Kurrasch, 2018). This microglial activation can impact neural programing and circuitry, and ultimately result in behavioural deficits later in life (Schaafsma et al., 2017; Pratt et al., 2013; Williamson et al., 2011; Diz-Chaves et al., 2012; Kelley et al., 2017).

Microglia ablation studies using colony-stimulating factor-1 receptor (CSF1R) inhibitors have demonstrated that microglia possess unique properties for a CNS cell. Most notable is the observation that they can repopulate (from ~99% depletion) in the postnatal or adult brain within one week of removal of the CSF1R inhibitor (Rosin et al., 2018; Elmore et al., 2014). Although newly repopulated microglia were originally thought to arise from CNS cells of a neural lineage (Elmore et al., 2014), more recently it has been suggested that microglia repopulate from the self-renewal of the limited number of microglia that remain in the CNS following depletion (Huang et al., 2018). This finding is supported by studies showing that across our lifetime microglia self-renew and replenish their resident populations from the proliferation of microglia residing in the CNS (Askew et al., 2017). Indeed, in both mice and humans, this observed rapid turnover of microglia in the adult brain suggests that our entire microglia population may be renewed several times during our lifespan (Askew et al., 2017). Together, these results are beginning to shed light on the importance of microglia for the development and continued function of a normal and healthy CNS across our life.

In the hypothalamus, a role for microglia in the development of key energy centers is emerging. For example, depletion of fetal microglia specifically during gestation using the CSF1R inhibitor PLX5622 causes a decrease in POMC neurons postnatally and a concomitant accelerated weight gain in early postnatal life (Fig. 2) (Rosin et al., 2018). Interestingly, leptin receptors are not expressed in embryonic hypothalamic microglia (Rosin et al., 2018), despite being expressed in adult microglia (Fig. 1) (Chang et al., 2017) and having been associated with hyperphagia and weight gain independent of neuronal leptin receptors (Gao et al., 2018). This lack of leptin signaling in embryonic microglia supports the notion that the impact of the loss of microglia in the developing hypothalamus might be due to directly affecting the establishment of specific populations of neurons. This early and striking postnatal weight gain is unique amoung animal models with impaired hypothalamic signaling. Indeed, the change in body mass following a hypothalamic insult (best example is leptin deficient ob/ob mice) is typically only observed starting late postnatally and into adulthood (Gao et al., 2018; Urabe et al., 2013; Gao et al., 2017; Valdearcos et al., 2017; Yi et al., 2017; Gao et al., 2014); in contrast, the loss of fetal microglia causes an obese phenotype starting by postnatal day 5 (P5) (Fig. 2) (Rosin et al., 2018), suggesting a key role for microglia in development of hypothalamic circuits. Intriguingly, a recent study highlights a unique phenomenon that involves microglia and occurs in the ARC of the developing hypothalamus (Lutz and Le Foll, 2019). Specifically, the peptide hormone amylin, which influences neurogenesis, axonal fiber outgrowth, and leptin signaling in the hypothalamus (Abegg et al., 2017; Liberini et al., 2016; Lutz et al., 2018) also appears to be involved in the birth of microglia in the ARC (Lutz and Le Foll, 2019). This suggests that microglia that reside in the hypothalamus during embryogenesis and early postnatally may indeed represent spatially unique populations that signal and respond to the local microenvironments present in each of the nuclei within the hypothalamus.

Moreover, it is already becoming clear that hypothalamic neurons

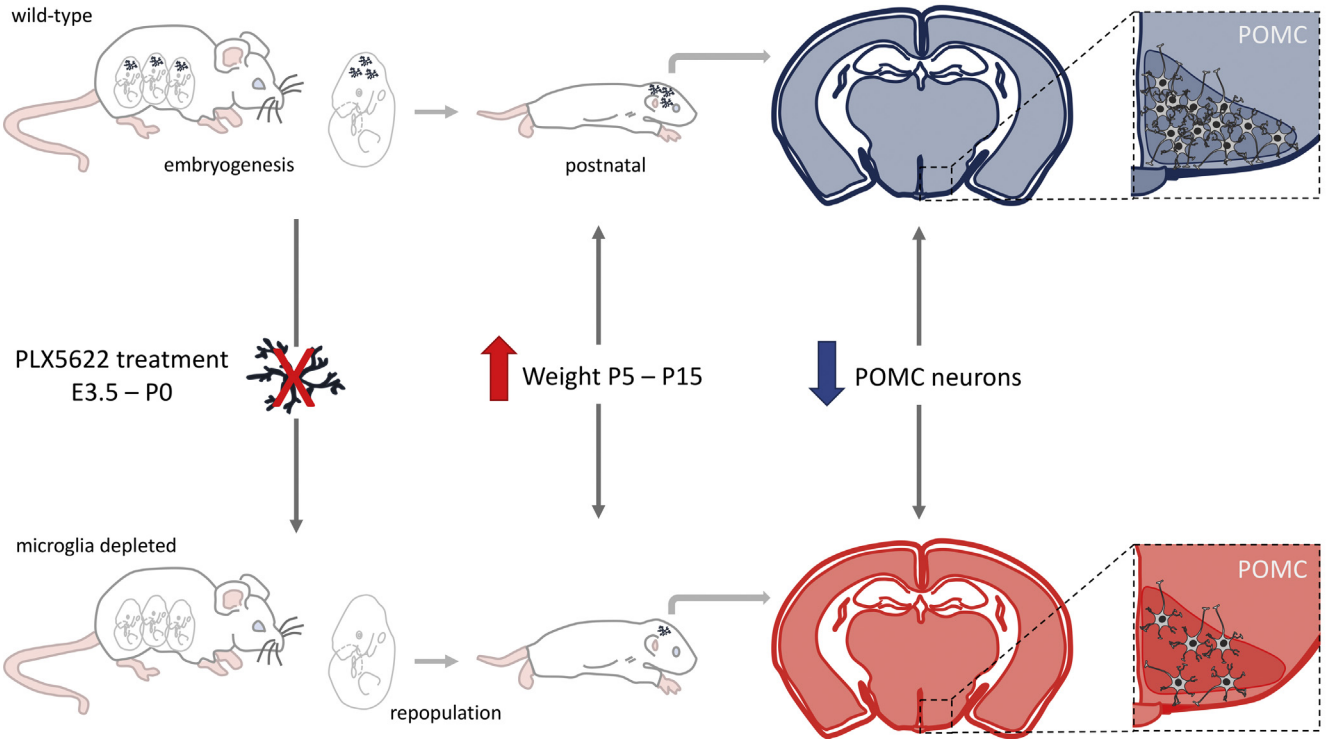


**Fig. 2.** Depletion of embryonic microglia using the CSF1R inhibitor PLX5622 results in accelerated weight gain and a concomitant reduction in POMC neurons. Schematic diagram of the effects of treatment of pregnant dams with the colony-stimulating factor-1 receptor (CSF1R) inhibitor PLX5622 from embryonic day 3.5 (E3.5) to birth (postnatal day 0, P0). On the top and bottom of the diagram, embryos and pups are depicted with or without microglia (dark blue cells), respectively, in their central nervous system (CNS), depending on whether the pregnant dams from which they were born were treated with PLX5622. Mouse pups born to dams treated with PLX5622 during pregnancy show an increase in weight gain (red arrow) from P5 through to P15. Cartoon brain slices to the right illustrate coronal sections through the P4 hypothalamus of control (blue) and microglia depleted (red) pups. To the far right, higher magnification cartoon images illustrate coronal sections through the arcuate nucleus (ARC) of control (blue) and microglia depleted (red) P4 pups, and show a decrease (blue arrow) in pro-opiomelanocortin (POMC) neurons (grey cells) in the ARC of microglia depleted (red) pups as compared to control (blue) pups. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and microglia work together to coordinate and manage metabolic physiology. In the adult mouse brain, microglia-related phenotypes impacting hypothalamic energy balance signaling have been demonstrated across various animal models, collectively suggesting that microglia and neighboring hypothalamic neurons likely interact to control feeding behaviours. For example, mice that lack lipoprotein lipase activity specifically in microglia gain weight and show a reduction in POMC neuronal numbers when challenged with a high-carbohydrate high-fat (HCHF) diet (Gao et al., 2017). Moreover, mice with a loss of brain-derived neurotrophic factor (BDNF) specifically in hematopoietic cells become obese and develop insulin resistance and hyperphagia (Urabe et al., 2013). Furthermore, inhibition of microglia via minocycline administration results in increased food intake (Reis et al., 2015). Together, these studies strongly support the idea that microglia play a key role in the regulation of energy homeostasis, despite the underlying signaling interaction pathways being poorly characterized.

Some clues into how microglia might communicate with neighboring hypothalamic neurons can be found by examining the surface receptors present on microglia. For example, microglia cells use toll-like receptors (TLRs) and the tyrosine kinase receptors tyro/axl/mer (TAM) to detect pathogens (Fig. 1) (Tufail et al., 2017, reviewed in ref. Kettenmann et al., 2011). Indeed, recent studies demonstrate that TLRs play a role in hypothalamic neuroinflammation, metabolic dysfunction, and obesity (Zhang et al., 2008; Benzler et al., 2015; Schaeffler et al., 2009). Similarly, the release of adenosine diphosphate/triphosphate (ADP/ATP) from injured cells can be sensed by microglial purinergic receptors, while microglia can also use sialic-acid-binding immunoglobulin-like lectins (SIGLEC) to detect sialic acids (reviewed in ref. Garcia-Caceres et al., 2019; Kettenmann et al., 2011). Perhaps, these same receptors are also involved in microglial sensing of physiological changes involved in homeostatic imbalances. Indeed, a recent study found that the canonical B cell receptor cluster of differentiation 22 (CD22), which mediates the anti-phagocytic effect of α2,6-linked sialic acid, is upregulated on aged microglia (Pluvinage et al., 2019). Moreover, by blocking CD22 microglial phagocytosis can be restored to homeostatic levels within the aging brain (Pluvinage et al., 2019). Further still, microglia are not limited to detecting these few ligand/receptor signaling pathways, as they express receptors for numerous neurotransmitters and cytokines/chemokines (Fig. 1) (reviewed in ref. Le Thuc et al., 2017; Kettenmann et al., 2011), with a variety of cytokine/chemokine receptors showing specific roles in microglial signaling during hypothalamic neuroinflammation and metabolic dysfunction (reviewed in ref. Le Thuc et al., 2017; Avalos et al., 2018). Considering the critical roles microglia play during hypothalamic development and function, especially in regulating food behaviours and energy expenditure, understanding the interaction between hypothalamic microglia and neuronal populations will likely contribute to our understanding of the etiology of neuroendocrine disorders, such as obesity.

## 4. Microglial involvement in hypothalamic neuroinflammation

If microglia themselves act as sensors to influence hypothalamic control of homeostasis, then mechanistically how do microglia sense these peripheral cues? In general, the brain and periphery have adopted several unique pathways of communication, with neural and humoral pathways both being involved in brain-periphery communications. Specifically, the vagus nerve along with the dorsal vagal complex, and its unique cytokine receptors, are involved in signaling through the neural pathway (Williams et al., 2000, reviewed in ref. Le Thuc et al., 2017; Grijalva and Novin, 1990). The humoral pathway, in contrast, involves the circulation of cytokines that become elevated during times of inflammation. Cytokines can use a variety of pathways to signal to the brain, including receptor binding to endothelial cells or increased blood-brain barrier (BBB) permeability during inflammation, which further allows cytokines to reach circumventricular organs and the choroid plexus (Wispelwey et al., 1988, reviewed in ref. Le Thuc et al., 2017). In addition to the neural and humoral pathways, infiltrating immune cells can invade the brain during times of inflammation. Although microglia have been shown to mediate inflammatory processes in the brain, the exact mechanism by which microglia receive cues that are involved in hypothalamic neuroinflammation is an active area of research.

A growing body of evidence now links body weight changes to hypothalamic neuroinflammation. For example, in humans, a correlation between obesity and glial activation in the hypothalamus exists (Schur et al., 2015), whereas in mice, involuntary weight loss *and* weight gain are both causally linked to hypothalamic neuroinflammation that presumably similarly disrupts feeding behaviours and energy balance (reviewed in ref. Le Thuc et al., 2017; Avalos et al., 2018; Garcia-Caceres et al., 2019). Moreover, an increased incidence of diabetes and obesity is observed in offspring following maternal consumption of a high-fat diet (HFD) during gestation and lactation (Nivoit et al., 2009), and likewise, children born to obese mothers can develop hyperphagia, resulting in increased weight, higher fat percentage, and insulin resistance (Nivoit et al., 2009; Samuelsson et al., 2008, reviewed in ref. Ornoy, 2011; Tamashiro and Moran, 2010). These findings suggest that early life exposure to maternal metabolic challenges somehow alters hypothalamic developmental programs that result in neuroendocrine consequences later in life. Here we propose that fetal microglia are a sensor for this maternal metabolic state and are ultimately responsible for transmitting these cues to the developing hypothalamus. Studies investigating metabolic control and energy balance postnatally and in the adult highlight an intriguing dichotomy, in that low levels of hypothalamic neuroinflammation is associated with obese phenotypes, while more severe inflammation appears to result in involuntary weight loss (Braun and Marks, 2010, reviewed in ref. Le Thuc et al., 2017; Avalos et al., 2018; Velloso et al., 2008). Below we profile these two opposite neuroinflammatory states and discuss how microglia might be equally responsive in mediating them.

### 4.1. Chronic low-grade hypothalamic neuroinflammation

Chronic low-levels or low-grade hypothalamic neuroinflammation is often associated with obesity. Early work examining diet-induced hypothalamic neuroinflammation in rats following exposure to HFD describe an elevation in hypothalamic pro-inflammatory cytokines, including tumour necrosis factor alpha (TNFα), interlukin-1 (IL-1), and interlukin-6 (IL-6) (De Souza et al., 2005). Moreover, activation of c-Jun N-terminal kinases (JNKs), nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), and endoplasmic reticulum stress (Fig. 1), in addition to leptin and insulin signaling impairments, are also consistently reported in diet-induced hypothalamic neuroinflammation studies (Zhang et al., 2008; Benzler et al., 2015; De Souza et al., 2005; Kleinridders et al., 2009). Although NF-κB activation is most often associated with pathogenic insults and pro-inflammatory cytokine responses involving TLRs, hypothalamic neuroinflammation and obesity are surprisingly linked to NF-κB activation. For example, activation of hypothalamic NF-κB, potentially via microglia TLRs, interferes with normal leptin/insulin signaling, while NF-κB suppression within the mediobasal hypothalamus, perhaps via microglia or in AgRP neurons, can be protective against glucose intolerance and obesity following over-feeding (Zhang et al., 2008; Benzler et al., 2015). Indeed, recent studies show that saturated fatty acids (SFAs), which are recognized by the mediobasal hypothalamus and involved in energy homeostasis, are abundant in HFD and can activate NF-κB via TLR4 *in vitro* (Fig. 1) (Schaeffler et al., 2009). Moreover, *in vivo* studies in rodent models show that long-chain SFAs activate TLR4 signaling in the hypothalamus, which results in the induction of cytokine expression (e.g., TNFα, IL-1β, IL-6) and ER stress (Milanski et al., 2009). Further yet, rats fed a HFD rich in monounsaturated fats do not develop leptin resistance, while inhibiting TLR4 signaling in mice, specifically through TLR4 mutation or pharmacological inhibition, can prevent diet-induced

obesity (Milanski et al., 2009). Similarly, palmitic acid, a common SFA found in fats, is detected in the hypothalamus and can result in hypothalamic neuroinflammation comparable to that observed following HFD exposure (Morselli et al., 2014; Valdearcos et al., 2014), further suggesting that microglia might be responsive to these SFAs in the hypothalamus. However, it should be noted that while TLR4 does appear to be involved in long-chain SFA induced inflammation, which has been proposed to occur through TLR4-dependent priming and altered cellular metabolism, TLR4 is not a receptor for the long-chain SFA palmitate (Lancaster et al., 2018). Although the mechanism of SFA action is still an active area of research, SFAs could directly be responsible for diet-induced neuroinflammation, especially when SFA consumption is high, such as in the case of high-fat foods often classified as the "Western diet". Together, these findings suggest that specific nutrient excess, such as high-levels of SFA, may be one of the major contributors to hypothalamic neuroinflammation, and that inappropriate NF-κB activation may be a mechanism through which the energy imbalance underlying obesity is achieved. Therefore, the suppression of improper NF-κB activation may represent one strategy to therapeutically target obesity and related neuroendocrine diseases.

Considering that microglia activation gives rise to hypothalamic neuroinflammation prior to the onset of obese phenotypes (e.g., is apparent within days of HFD exposure in both rats and mice), it is possible that microglial neuroinflammation in the hypothalamus itself could be the upstream instigator of metabolic disturbances and excess weight gain (Thaler et al., 2012, reviewed in ref. Le Thuc et al., 2017; Avalos et al., 2018). In support of this notion, recent studies demonstrate that blocking hypothalamic neuroinflammation impedes metabolic disruption and obesity (Valdearcos et al., 2017; Zhang et al., 2008; Dorfman et al., 2017). Indeed, shortly after HFD exposure, microglia appear to act as the first responders in the hypothalamus, with microglia displaying an activated phenotype and being both recruited to and proliferating within the mediobasal hypothalamus (Valdearcos et al., 2014; Thaler et al., 2012). Furthermore, microglia are thought to be the first cells that alter their morphology and physiological properties following obesogenic diet exposure, which is supported by *in vivo* microglial depletion studies in mice that demonstrate a reduction of food consumption and hypothalamic neuroinflammation in the absence of microglia (Valdearcos et al., 2014; Thaler et al., 2012). In contrast, complementary experiments demonstrate that neuroinflammation and microglia-specific NF-κB signaling are required for diet-induced obesity (Valdearcos et al., 2017). Specifically, loss of microglial NF-κB signaling in IKKβ knockout animals causes a decline in food intake and weight gain, while activation of microglia NF-κB signaling, through deletion of the negative NF-κB regulator A20, results in an increase in obesity even in the absence of HFD (Valdearcos et al., 2017). Combined, these studies show that microglia play a central role in alterations in hypothalamic energy balance.

Mechanistically, activated microglia might communicate with nearby hypothalamic neurons via CX3CL1, a chemokine that is thought to restrict microglia activation. Interestingly, CX3CL1 expression is induced in hypothalamic neurons of an obesity-prone mouse strain (i.e., Swiss mouse) following HFD exposure (Morari et al., 2014). Moreover, inhibiting CX3CL1 in the hypothalamus reduces HFD-induced neuroinflammation, and appears to protect mice against glucose intolerance (Morari et al., 2014). Similarly, hypothalamic CX3CL1 expression is elevated in fructose-fed mice that showed microglial activation (Xu et al., 2016). In contrast, overexpression of CX3CL1 limits hypothalamic microglia activation and blocks diet-induced obesity following HFD feeding (Dorfman et al., 2017, reviewed in ref. Le Thuc et al., 2017; Sheridan and Murphy, 2013). This signaling relies on TNFα, which becomes reduced following CX3CL1 overexpression and can lessen diet-induced obesity (Yi et al., 2017). Therefore, during HFD exposure, cross-talk between microglia and neurons may be altered through a reduction in CX3CL1, which would ultimately increase microglia activation and elevate TNFα secretion and pro-inflammatory signaling, leading to changes in energy balance and the onset of obesity. Although it is clear that hypothalamic neurons and microglia can both influence metabolic physiology, it is not clear which of these populations act first to initiate the programs that will ultimately result in dysregulation and obesity. Indeed, both neurons and microglia express a number of pro-inflammatory cytokines/chemokines (reviewed in ref. Le Thuc et al., 2017; Sheridan and Murphy, 2013) and disrupting either population can result in altered metabolic states (Rosin et al., 2018; Gao et al., 2018; Gao et al., 2017; Yi et al., 2017; Hill et al., 2009; Coupe et al., 2012). Moreover, it is not clear what the exact signal or upstream instigator of metabolic disturbances and excess weight gain is, making the hierarchical classification of the specific cell type responsible for the initiation of metabolic dysregulation challenging. Furthermore, the inflammatory response that results from a hypercaloric environment appears to be temporally regulated by the exposure period of HFD. Specifically, a daily rhythm to microglia reactivity in mice has been proposed, with this diurnal activity lost during diet-induced obesity leading to increased microglia numbers and reactivity even after four months on a HCHF diet (Yi et al., 2017). These findings support a persistent elevation in microglial reactivity in high caloric states (Yi et al., 2017). Similarly, continued HFD feeding results in permanent mediobasal hypothalamic neuroinflammation (Thaler et al., 2012; Berkseth et al., 2014). In contrast, the effects of HFD feeding appear to be transient and reversible if mice originally fed a HFD are placed on regular chow (Thaler et al., 2012; Berkseth et al., 2014). Although the action of HFD in chronic low-grade hypothalamic inflammation and obesity is still an active area of study, together these findings indicate that microglial activation and neuroinflammation play prominent roles in diet-induced obesity following HFD exposure. In general, hypothalamic microglia appear to be affected by diet, adipokines, and perhaps gut signals, leading to elevated pro-inflammatory signaling, which has been consistently shown to act on neurons to dysregulate metabolic processes and energy balance.

### 4.2. Severe hypothalamic neuroinflammation

In contrast to low levels of inflammation, severe hypothalamic neuroinflammation can cause involuntary weight loss (reviewed in ref. Le Thuc et al., 2017; Avalos et al., 2018). Generally speaking, involuntary weight loss, such as in the case of inflammation-induced anorexia or sickness behaviour in rodents, usually involves an elevation in pro-inflammatory cytokines. Rodents used to model the phenotypes associated with involuntary weight loss are often administered bacterial lipopolysaccharides (LPS) or given systemic infection to organs peripheral to the CNS (reviewed in ref. Alexander and Rietschel, 2001). Severe inflammation can also occur during local CNS infection or injury, with studies showing that even the immature embryonic hypothalamus can become severely inflamed following infection (Rosin and Kurrasch, 2018). Specifically, *in utero* electroporation (IUE), a gene transfer technique that induces the uptake of foreign DNA into progenitor cells that line the ventricles, triggers an immune response causing a dramatic increase in the number of activated microglia, both via upregulation of CD45 and downregulation of the purinergic receptor P2RY12 (Rosin and Kurrasch, 2018). Indeed, this procedure also results in an elevation in pro-inflammatory cytokines, including TNFα, IL-1β, and IL-6, amoung others (Rosin and Kurrasch, 2018), which is consistent with an activated microglial state, and the subsequent infiltration of peripheral immune cells (Tufail et al., 2017; O'Loughlin et al., 2017; Giulian et al., 1986, reviewed in ref. Dickson et al., 1993). Given that the mediobasal hypothalamus is so-called leaky with unique milieus, and that cytokines can use extracellular diffusion to cross the vasculature/BBB to easily move throughout the brain parenchyma, blood vessels/bloodstream, and into cerebrospinal fluid (CSF), it is not surprising that damage to the hypothalamus resulting from infection or injury can become widespread across the CNS (Gotow and Hashimoto, 1981, reviewed in ref. Vitkovic et al., 2000; McAdams and Juul, 2012).

Studies into the consequences of severe hypothalamic neuroinflammation has led to focused attention on the pro-inflammatory cytokine IL-1, since it plays a key role in the development of behavioural changes in rodents. Specifically, intracerebroventricular injection of IL-1α or IL-1β reduces exploratory behaviour in mice (Spadaro and Dunn, 1990). Moreover, IL-1β may mediate LPS-induced anorexia and act to regulate cytokine release and signaling in the hypothalamus, since IL-1β can directly act on POMC neurons in the ARC (Scarlett et al., 2007). Furthermore, vagotomy experiments demonstrate that the vagus nerve plays a role in LPS/IL-1-mediated signaling to the hypothalamus, limbic system, and brainstem during the onset of sickness behaviour (reviewed in ref. Le Thuc et al., 2017; Konsman et al., 2002). This is an interesting finding given that IL-1 producing immune cells are present in the perineural sheath surrounding the vagus nerve, and that IL-1 receptors are present on vagus nerve sensory neurons, which can be stimulated by IL-1 (reviewed in ref. Le Thuc et al., 2017; Konsman et al., 2002). Finally, a recent report, using a genetic knock-in reporter system to express or delete interleukin-1 receptor 1 (IL-1R1) in mice, shows that IL-1R1 is involved in leukocyte and monocyte recruitment to the CNS and is necessary to mediate sickness behaviour (Liu et al., 2019). Although microglia are not responsive to IL-1, since they do not express IL-1R1, microglia produce IL-1 (Liu et al., 2019). Therefore, these studies provide a link between IL-1-producing microglia and severe hypothalamic neuroinflammation, resulting in behavioural consequences such as anorexia and sickness behaviour.

As previously discussed, TLRs are involved in hypothalamic neuroinflammation and changes in feeding behaviour. In the context of severe hypothalamic neuroinflammation, LPS-induced sickness behaviour results, in part, from microglia activation through TLR4, since disruptions to TLR4 activity prevents microglia activation, cytokine release, and the onset of sickness behaviours (Hines et al., 2013; Lien et al., 2000, reviewed in ref. Gay et al., 2006). Similarly, a TLR2 agonist can also induce microglial activation and the development of sickness behaviour, which involves both NF-κB and cyclooxygenase (COX) signaling (Jin et al., 2016). Moreover, TLR2 activation increases microglia occupancy of the ARC and leads to: 1) a decrease in gamma-aminobutyric acid (GABAergic) contacts on POMC neurons and 2) an increase in vesicular glutamate transporter 2 (vGLUT2) contacts on POMC neurons, which together results in an increased excitatory state (Jin et al., 2016). Although microglial TLR2 activation can induce sickness behaviours, C-C chemokine ligand/receptor type 2 (CCL2/CCR2) is required for the negative metabolic and behavioural consequences that develop following LPS exposure. Specifically, LPS-induced involuntary weight loss is diminished in CCR2 knockout animals (Le Thuc et al., 2016). Similarly, the use of antagonists against CCR2 following LPS exposure also results in decreased weight loss when compared to animals exposed to LPS injection alone (Le Thuc et al., 2016). Together, these studies suggest that significant levels of microglial activation and hypothalamic neuroinflammation can negatively impact neuropeptidergic circuitry, and ultimately lead to involuntary weight loss.

## 5. Microglial priming and the inflammatory effects of stress

Another factor that can act to induce hypothalamic neuroinflammation and disrupt homeostatic balance is stress. In this section, we outline evidence that the neurobiology of stress might involve microglia as an intermediary. Stress, whether it be acute or chronic, can activate the hypothalamic-pituitary-adrenal (HPA) axis and result in the release of stress hormones, such as glucocorticoids, from the adrenal gland. Moreover, systemic elevation of glucocorticoids following stress has been implicated in various damaging scenarios, such as neuroinflammation and tissue damage. Indeed, studies show that glucocorticoids mediate stress-induced microglia priming and neuroinflammation (Fig. 1) (reviewed in ref. Frank et al., 2015; Frank et al., 2019; Sorrells and Sapolsky, 2007). Specifically, microglia isolated from rodent brains following stress did not become primed when treated with LPS if the microglia were pre-treated with mifepristone (glucocorticoid receptor antagonist) or were isolated from adrenalectomized mice (Frank et al., 2010), supporting a role for glucocorticoids in stress-induced microglial priming. Similarly, acute or chronic exposure of isolated microglia to exogenous glucocorticoids induces pro-inflammatory signaling by microglia, further supporting a role for glucocorticoids in priming microglia (Frank et al., 2010; Munhoz et al., 2010). Interestingly, administering glucocorticoids prior to immune challenge (e.g., two or twenty-four hours prior) results in pro-inflammatory signaling, while exposure to glucocorticoids following immune challenge (e.g., one hour following) causes suppression of the pro-inflammatory response induced by the challenge (Frank et al., 2010). Given that glucocorticoids have been widely used as anti-inflammatory drugs, these and other studies are beginning to suggest that the time at which the body is exposed to glucocorticoids, relative to the immune challenge, may be an important determinant as to whether glucocorticoids exhibit pro- or anti-inflammatory effects (Frank et al., 2010; Munhoz et al., 2010, reviewed in ref. Frank et al., 2019). Combined, the role microglia play in mediating the hypothalamic stress response is still emerging.

Catecholamines, such as epinephrine, are also produced by the adrenal glands and are involved in the stress response (reviewed in ref. Frank et al., 2019). Similar to glucocorticoids, catecholamines mediate the inflammatory signaling associated with stress (Weber et al., 2017; Johnson et al., 2005; Johnson et al., 2013). Moreover, catecholamines are also thought to play a role in stress-induced microglial priming. Specifically, studies examining the negative consequences of repeated social defeat show that propranolol (β-adrenergic receptor antagonist) treatment inhibits upregulation of ionized calcium binding adaptor molecule 1 (IBA1) and IL-1β in microglia throughout a number of regions in the brain (Wohleb et al., 2011), consistent with other studies demonstrating stress directly impacts microglia. In contrast, exposure to isoproterenol (β-adrenergic agonist) primes microglia and results in neuroinflammation resembling that seen following stress (Johnson et al., 2005; Johnson et al., 2013). Together, these studies support a role for catecholamines in stress-induced microglial priming and neuroinflammation mediated through β-adrenergic receptor signaling.

Catecholamines also help recruit peripheral immune cells, such as monocytes and inflammatory macrophages to the CNS (Weber et al., 2017; Wohleb et al., 2011); however, only recently has the role of microglia in this recruitment become clear (McKim et al., 2018). Specifically, stress-induced microglia play a role in the recruitment of monocytes to the CNS using CCL2/CCR2 signaling (McKim et al., 2018). Moreover, stress-induced monocyte recruitment to the CNS elevates pro-inflammatory IL-1β signaling in the brain parenchyma, which plays a prominent role in behavioural consequences observed following stress (McKim et al., 2018), thereby positioning microglia as a central player in the stress response. Although increased IL-1β release following stress has been implicated in behavioural impairments, glucocorticoids and catecholamines are thought to act in opposition to regulate IL-1β production. Glucocorticoids can inhibit IL-1β release via glucocorticoid receptors, while catecholamines stimulate IL-1β release by signaling through β-adrenergic receptors (Barnard et al., 2019). Recently, studies have begun to question how the release of two stress-induced molecules – glucocorticoid and catecholamine – can differentially act to regulate IL-1β production. Intriguingly, this response is sexually dimorphic since male and female rats exposed to stress for four days, followed by treatment with metyrapone (glucocorticoid synthesis inhibitor), propranolol (β-adrenergic receptor antagonist), or both, showed differing levels of IL-1β mRNA (Barnard et al., 2019). Specifically, treatment with propranolol post-stress results in reduced IL-1β levels in chronically stressed male and female rats (Barnard et al., 2019). In contrast, metyrapone treatment following stress causes increased IL-1β levels throughout the brain in chronically stressed male rats, while female rats show a specific increase in the hypothalamus (Barnard et al., 2019). Moreover, microglia are shown to directly contribute to increased IL-1β production following metyrapone treatment (Barnard et al., 2019).

Interestingly, treatment with both propranolol and metyrapone block the elevation in IL-1β levels, suggesting that the increased IL-1β levels post-metyrapone treatment likely result from β-adrenergic receptor activation (Barnard et al., 2019). As a whole, while glucocorticoids and catecholamines may act through different and opposing pathways to regulate IL-1β production, these studies strongly support roles for stress hormones in microglial priming, neuroinflammation, and the resulting behavioural impairments that accompany stress.

### *5.1. A role for danger-associated molecular patterns (DAMPs) in stress-induced neuroinflammation*

Danger-associated molecular patterns (DAMPs) are proteins found within the cell under normal physiological conditions, which then become released from the cell when it is stressed or dying. Immune cells express receptors for DAMPs on their surface and go on to initiate pro-inflammatory signaling and launch an immune response following recognition of DAMPs (Fig. 1). Given that glucocorticoids can induce neuronal stress and cell death (reviewed in ref. Sapolsky, 1999), it is perhaps not surprising that a downstream effects of stress is a DAMP-induced neuroinflammatory response (reviewed in ref. Frank et al., 2019). Indeed, stress can elevate the levels of DAMPs in the CNS (Weber et al., 2015; Iwata et al., 2016; Lian et al., 2017) and DAMPs play a role in microglial priming (Weber et al., 2015). Specifically, microglia isolated from Sprague Dawley rats exposed to a tail shock and injected with a high mobility group box 1 (HMGB1, a DAMP) antagonist, do not display a pro-inflammatory response following LPS treatment (Weber et al., 2015). Similarly, administration of the pro-inflammatory form of HMGB1 itself can prime microglia (Frank et al., 2016). This demonstrates that DAMP signaling is involved in stress-induced microglia priming and neuroinflammation, further linking microglia activation to homeostatic processes.

Intriguingly, microglia isolated from Sprague Dawley rats exposed to a tail shock release HMGB1 (Weber et al., 2015), implying that microglia themselves are a source of DAMP release in response to stress. Mechanistically, this microglia-induced DAMP release is thought to be achieved by cluster of differentiation 200 ligand/receptor (CD200/CD200R1) signaling, since both tail shock (e.g., stress) and glucocorticoid exposure results in the down-regulation of CD200R1 specifically on microglia (Fonken et al., 2016; Frank et al., 2018; Frank et al., 2018). Moreover, injecting a CD200R1 agonist prior to stress blocks microglia priming and HMGB1 levels in the brain, suggesting that by down-regulating CD200R1, stress primes microglia and causes the release of HMGB1 (Frank et al., 2016). Accordingly, CD200/CD200R1 appears to act similar to CX3CL1/CX3CR1, whereby CD200 is expressed on neurons and is thought to inhibit microglia by means of CD200R1 in order to preserve microglia surveillance and homeostasis under normal conditions (Koning et al., 2009; Wright et al., 2000, reviewed in ref. Frank et al., 2019; Gorczynski, 2005; Deczkowska et al., 2018). Considering that HMGB1 can bind to IL-1 and amplify IL-1 signaling (reviewed in ref. Yang et al., 2015), these studies show that DAMPs also contribute to stress-induced microglial priming and neuroinflammation, and may act together with IL-1 to yield the microglia-influenced behavioural consequences that are observed following stress.

## 6. Hypothalamic microglia and the microbiome

Recent scientific advancements highlight the importance of the gut-brain axis and the microbiome, the microorganisms that colonize our body, to overall health. The specific interactions between the microbiome and the hypothalamus are being uncovered (Fig. 1). For example, the role of fatty acids in the onset of obesity through microbiota-driven inflammation has been examined (Zhuang et al., 1863). Indeed, arachidonic acid favours pro-inflammatory microbiota, which causes microglia-induced leptin resistance through TLR4 and NF-κB signaling and global inflammation in male mice (Zhuang et al., 1863). Other groups have focused their studies on the transition from a largely sterile environment *in utero* to the dramatic elevation in microbiota exposure that occurs at and following birth (Castillo-Ruiz et al., 2018). Specifically, mice born in germ-free (GF) conditions display a reduction in the pro-inflammatory cytokines TNF-α and IL-1β, as well as an increase in the number and size of microglia (Castillo-Ruiz et al., 2018). Moreover, higher rates of cell death in the PVN and lower numbers of cell death in the ARC are observed in the hypothalamus of mice born in GF conditions (Castillo-Ruiz et al., 2018), suggesting that microbiota exposure at birth plays specific roles in hypothalamic nuclei development, in addition to contributing to normal microglial functions. Furthermore, microglial activation in the PVN of the hypothalamus influences hypertension in rats, with concomitant changes in gut microbiota also reported (Sharma et al., 2019), suggesting a link between microglia and microbiota, whereby microglia may signal and communicate with the microbiota in the gut during sustained hypertension. Interestingly, the vast majority of studies that have investigated the influence of the microbiome on hypothalamic and other CNS functions are reporting striking sex differences, particularly involving microglial responses (Zhuang et al., 1863; Thion et al., 2018); suggesting additional layers of complexity beyond that already portrayed by microbiome research. Although research is still in its infancy, an accumulation of evidence now posits that hypothalamic microglia are exquisitely sensitive to gut-brain axis cues.

## 7. Microglia are both sexually dimorphic and contribute to sexual dimorphism in the hypothalamus

A number of studies now reveal that microglia have sex-specific roles across life (reviewed in ref. (Nissen, 2017). Although the fetal brain has yet to show sexual dimorphism in regard to microglia numbers and colonization, microglia in the postnatal brain display striking sex differences in their morphology, gene expression, colonization, inflammatory activation, and response to drugs (Schwarz et al., 2012; Nikodemova et al., 2014; Mirza et al., 2015; Caetano et al., 2017). Specifically, there are more microglia in males as compared to females postnatally at P4, while females have a higher number of amoeboid microglia during juvenile and adult time-points (Schwarz et al., 2012). During neonatal hypoxic-ischemic encephalopathy (HIE), males demonstrate an increase in microglial activation and show an upregulation of inflammatory cytokines as compared to females, suggesting sexual dimorphism in the immune response following injury (Mirza et al., 2015). Similarly, prenatal exposure to the glucocorticoid dexamethasone, which is associated with the development of anxiety behaviours, results in the hyper-ramification of male microglia, while female microglia decrease in number and process length (Caetano et al., 2017). Moreover, following dexamethasone exposure, only males demonstrate a reduction in anxiety following treatment with an adenosine $A_{2A}$ receptor antagonist (Caetano et al., 2017). Combined, these studies indicate that microglia behave differently in the male and female brain.

Microglia also contribute to the sexual dimorphism that exists in the brain. Specifically, microglia are required for the masculinization of the preoptic area in the hypothalamus, which is known to contribute to male copulatory behaviours in adults (Lenz et al., 2013) and reviewed in ref. (Lenz and McCarthy, 2015; VanRyzin et al., 2018). Moreover, temporary depletion of microglia in rats using liposomal clodronate treatment during the early postnatal period (i.e., P0, P2, P4) results in deficits in male-specific sex behaviours during adulthood (VanRyzin et al., 2016). Furthermore, microglia knockout animals show hypothalamic-pituitary-gonadal axis disruption, further suggesting that the organization of neuronal connections in the hypothalamus are reliant on the presence and proper function of microglia during development (Cohen et al., 1997, reviewed in ref. Cohen et al., 1999) and consistent with work discussed above whereby a decrease in POMC neurons in the hypothalamus is observed in brains depleted of microglia during embryonic development (Rosin et al., 2018). Intriguingly, new

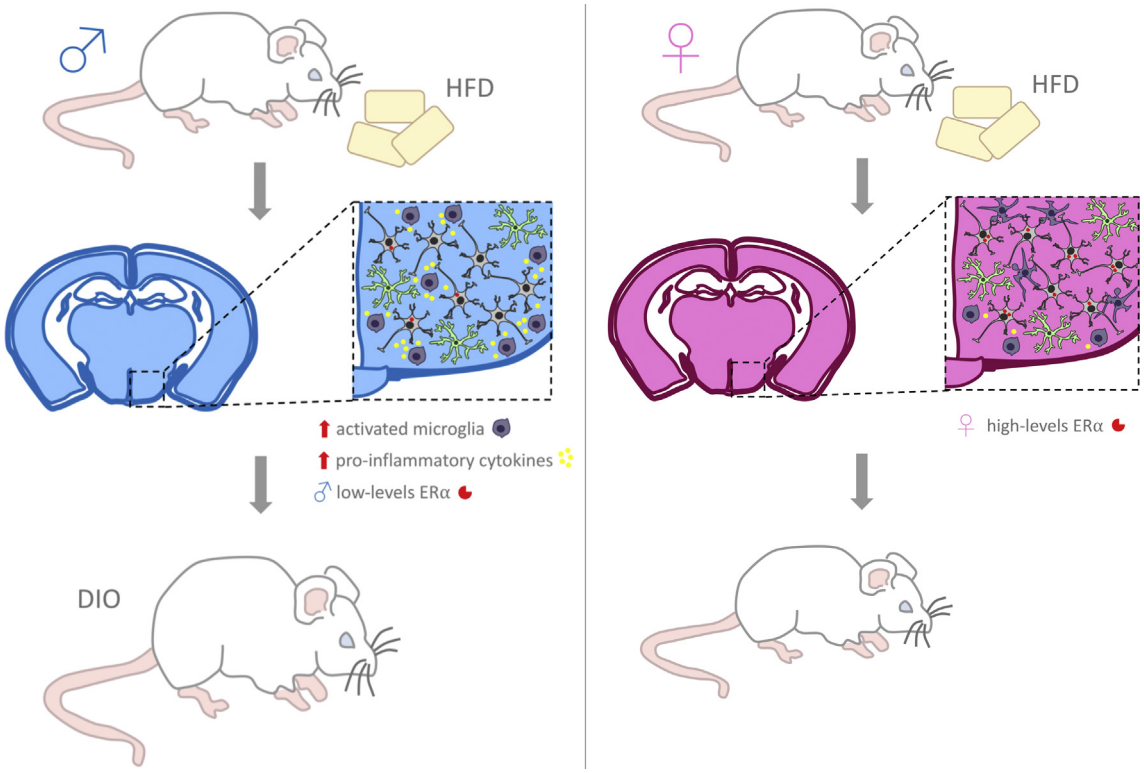


**Fig. 3.** Sexual dimorphism in low-grade inflammation and obesity. Schematic diagram showing that males (left) are more susceptible to hypothalamic neuroinflammation and developing diet-induced obesity (DIO) as compared to females (right). Top left, depicts treatment of a male mouse with high-fat diet (HFD). Middle left, cartoon hypothalamus (left) and high-magnification cartoon of the mediobasal hypothalamus (MBH, right) show that HFD treatment causes an increase in neuroinflammation, specifically an increase in the number of activated amoeboid microglia (purple cells) and an increase in the levels of pro-inflammatory cytokines (yellow circles). Bottom left, male mice become obese following HFD treatment. Top right, illustrates treatment of a female mouse with HFD. Middle left, cartoon hypothalamus (left) and high-magnification cartoon of the MBH (right) show that HFD treatment does not result in obvious hypothalamic inflammation, with relatively few activated amoeboid microglia (purple cells) present, and low levels of pro-inflammatory cytokines (yellow circles). Bottom left, female mice do not experience DIO following HFD treatment. Females may be protected from DIO because they express higher-levels of estrogen receptor alpha (ERα, red receptor) as compared to males. Astrocytes (green cells) and neurons (grey cells) are depicted in the high-magnification cartoons of the MBH for both males (left) and females (right). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and emerging work is also highlighting unique interactions between microglia and mast cells during development for both the normal onset of sexual behaviours during adulthood (Lenz et al., 2018), as well as during prenatal allergen exposure that can perturb sexual behaviours in adults (Lenz et al., 2019). Taken together, it appears that microglia can both be sexually dimorphic and contribute to the sexual dimorphism present in the brain, thereby suggesting that sexually dimorphic regulation of homeostatic processes might be driven by microglia that display sex-specific activation patterns.

### *7.1. Sexual dimorphism in low-grade inflammation and obesity*

Work from obesity rodent models repeatedly shows that sexual dimorphism exists in the response, development, and progression of neuroinflammation and phenotypic outcomes following a dietary challenge. For example, males are more susceptible to hypothalamic neuroinflammation and the onset of diet-induced obesity when compared to females (Fig. 3) (Morselli et al., 2014; Dorfman et al., 2017; Hong et al., 2009; Argente-Arizon et al., 2018). Therefore, not surprisingly, exposure to HFD results in an increase in the number of activated microglia and a greater elevation in pro-inflammatory signaling within the mediobasal hypothalamus in male mice compared to females (Fig. 3) (Morselli et al., 2014; Dorfman et al., 2017). Similarly, HFD exposure causes an increase in the detectable quantity of fatty acids in the brains of male mice, and male microglia are more activated in response to palmitic acid exposure (Morselli et al., 2014; Yanguas-Casas et al., 2018). The question then becomes, why does this sexual dimorphism exist (at least in rodents)? Interestingly, the observed sexual dimorphism apparent during dietary induction of hypothalamic neuroinflammation may be due to protection from estrogen, since both estrogen receptor alpha (ERα) and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) are down-regulated in neurons and astrocytes within the hypothalamus of male mice (Fig. 3) (Morselli et al., 2014). However, females can be pushed towards a phenotype resembling that of males following HFD exposure, specifically excess weight gain and hypothalamic neuroinflammation, if CX3CR1 signaling in microglia is disrupted using knockout models (Dorfman et al., 2017; Cardona et al., 2006). These findings suggest a protective role for both estrogen signaling in neurons and astrocytes and CX3CR1 in microglia, with these two pathways perhaps intersecting to protect females facing dietary challenges. Combined, this further strengthens the link between microglia and hypothalamic regulation of homeostatic processes.

In the general human population, no apparent differences in the prevalence of obesity exists between the sexes. This is consistent with correlative studies showing equal levels of hypothalamic gliosis in both male and female obese patients (Schur et al., 2015). Although no obvious differences in the phenotypic presentations appear to exist between obese men and women at present, sexual dimorphisms involving the onset of metabolic dysregulation and obesity are beginning to emerge. Specifically, metabolic disorders and obesity are less prevalent in premenopausal women as compared to men, however, metabolic dysfunction and obesity become dramatically elevated in women following menopause (Ford, 2005, reviewed in ref. Shi et al., 2009).

Moreover, men typically accumulate fat viscerally, while women carry a greater proportion of their fat subcutaneously (Kotani et al., 1994, reviewed in ref. Shi et al., 2009; Wajchenberg, 2000). This is a very important sex-dependent difference since the location of adipose tissue is a major factor in the health risks associated with obesity, with individuals carrying fat viscerally in their abdomen at a greater risk (reviewed in ref. Shi et al., 2009; Bjorntorp, 1997). Together, this suggests that men and women may have subtle differences in their regulation of energy homeostasis, likely involving hormones such as estrogen and perhaps even microglia signaling in the hypothalamus.

### *7.2. Sexual dimorphism in behaviour resulting from microglial disruptions*

As discussed throughout this review, a growing body of literature consistently highlights the importance of microglia for normal, healthy CNS development, and further illustrates a role for microglia as functional players contributing to the proper development of the embryonic, postnatal, and adult CNS. Although depletion of microglia during adulthood using the CSF1R inhibitor PLX2297 did not result in overt behavioural deficits (Elmore et al., 2014; Elmore et al., 2015), microglia depletion during embryonic development causes behavioural deficits in both adolescent and adult mice (Rosin et al., 2018). Specifically, adolescent female mice depleted of microglia during gestation are hyperactive using open field and elevated plus maze analysis. Intriguing, these behavioural deficits were not observed in adolescent male mice depleted of microglia during gestation, demonstrating a sex-specific effect of microglia on the developing brain (Rosin et al., 2018). Similarly, adult female mice depleted of microglia embryonically are less anxious than controls when analyzed using the elevated plus maze, while male mice that experienced microglia depletion during gestation show no change (Rosin et al., 2018). These findings are consistent with reports in CX3CR1 knockout mice, whereby disrupting microglia signaling through CX3CR1 results in hyperactivity, anxiolytic-like behaviours, and phenotypes resembling depression in mutant female mice only (Bolos et al., 2018). Combined, these results suggest that the presence of microglia are important for the development of key brain centers, which when disrupted can lead to lasting sex-specific behavioural consequences.

Although these studies demonstrate that embryonic microglia are important for specific behaviours in females, it is surprising that microglia depletion during gestation did not result in any overt behavioural consequences in male mice. This could reflect microglial sex differences, since microglia appear to mature across different developmental trajectories in males as compared to females (Bolton et al., 2017; Thion et al., 2018; Schwarz et al., 2012; Nelson and Lenz, 2017; Hanamsagar et al., 2018). Specifically, as discussed above, postnatal microglia show sex differences in their morphology, gene expression, colonization, and inflammatory activation (Schwarz et al., 2012; Nikodemova et al., 2014; Mirza et al., 2015; Caetano et al., 2017), with more microglia in males as compared to females early postnatally, although females have a higher number of amoeboid microglia later in life (Schwarz et al., 2012). Given the differences that exist in the developmental trajectory of microglia present in females as compared to males, subtle sex differences in microglia likely exist during embryogenesis. This could explain the behavioural differences observed between male and female mice following microglial depletion during gestation. Accordingly, future research should critically examine whether subtle differences, perhaps involving ligand/receptor expression, cell signaling, or estrogen responsiveness, for example, exist between male and female microglia during gestation.

## 8. Conclusions

In this review, we discuss recent evidence highlighting how microglia are responsive to external stressors, such as those external to the CNS (e.g., infection, stress, diet, gut-brain inputs, etc.) (reviewed in ref. Frank et al., 2015; Frank et al., 2019; Sorrells and Sapolsky, 2007; Rosin and Kurrasch, 2018; Thion et al., 2018; Le Thuc et al., 2017; Avalos et al., 2018; Garcia-Caceres et al., 2019), and how microglia can influence both the development and function of the hypothalamus. These studies support a strong interaction between microglia and hypothalamic neurons, whereby microglia may play an unappreciated role in hypothalamic control of physiological homeostasis, perhaps acting as upstream sensors to external inputs that then acts to modulate nearby hypothalamic neurons. We highlight the current literature demonstrating how microglia loss (e.g., depletion) or activation (e.g., inflammation) affects the hypothalamus, and ultimately body homeostasis. Specifically, we discuss how microglia sense dietary challenge (e.g., SFAs), infection (e.g., LPS), cytokines/chemokines (e.g., CX3CL1), stress (e.g., glucocorticoids, catecholamines, and DAMPs), and microbiota signals from the gut to influence hypothalamic development, circuitry, signaling, and neuroinflammatory states. Moreover, we highlight how microglia are both sexually dimorphic themselves and contribute to sexual dimorphism in the hypothalamus. Taken together, this literature supports our hypothesis that microglia might play an unappreciated role in homeostatic processes by sensing cues external to the CNS and transmitting them to neighboring hypothalamic neurons that can signal to regulate homeostasis.

Finally, microglia might be an unappreciated therapeutic target for the treatment of obesity, stress, and other homeostatic physiologies. By focusing much of our research attention on hypothalamic neuronal heterogeneity, signaling, and circuitry that underlies metabolic dysregulation and the onset of obesity, for example, we may have missed the benefit that modulating microglia presents. Now that microglia, as well as other neuronal–glial interactions such as neurons—astrocytes, are being highlighted as important players in systemic metabolism, perhaps pharmacological targeting to these glial cells represents an unappreciated avenue for drug discovery. Of course, achieving a balanced regulation of metabolic homeostasis is complex and might require intervention at various levels. Taken together, the findings discussed in this review strongly support a role for microglia in maintaining hypothalamic homeostatic processes, and suggests that microglia indeed could be key regulators of homeostasis by contributing to the development and continued function of the hypothalamus.

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